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Original Article

# Synthesis and Characterization of Some Impurities of Bisoprolol: Beta-Adrenoceptor Antagonist

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## ABSTRACT

**Aim/Background:** Throughout the drug development process, maintaining control over impurities and keeping them within specified limits is of paramount importance in the production of high-quality drugs. Numerous studies have been dedicated to synthesizing impurities and elucidating their structures to support the purification method. Bisoprolol, a selective beta-adrenoceptor antagonist that predominantly targets β1 receptors, is primarily employed to treat conditions like high blood pressure and angina. Therefore, it is crucial to synthesize and study impurities associated with Bisoprolol. The objective of this research work is to synthesize impurities of Bisoprolol, as these impurities play a critical role in the drug manufacturing process. Our research focused on the synthesis of 5 key impurities of Bisoprolol, along with their purification and characterization. **Experimental work:** These impurities were synthesized through processes such as Ring Opening, Esterification and Dimerization. The 5 impurities are as follows: 1. 1-(isopropyl amino)-3-(4-methylphenoxy) propan-2-ol, 2. 2-isopropoxyethyl4-(2-hydroxy-2-(isopropylmino)ethoxy) benzoate, 3. 4-[(2-Isopropoxyethoxy)methyl]phenol, 4. 3-[4-((2-Isopropoxyethoxy) methyl) phenoxy]-1, 2-propanediol, 5. 3, 3′-(isopropylazanediyl) bis (1-(4-((2- isopropoxyethoxy) methyl) phenoxy) propan-2-ol). **Characterization:** We employed column chromatography and thin-layer chromatography techniques to separate and purify these impurities. The structures of the synthesized compounds were determined using Infrared spectroscopy, Mass spectroscopy and 1H-NMR spectroscopy. The pharmaceutical industry, reducing impurity levels to meet the required thresholds by ICH guidelines holds promise for the future. **Conclusion:** by synthesizing impurities related to Bisoprolol, an emergency cardiovascular drug, our research contributes to the pharmaceutical industry's efforts to ensure the safety and efficacy of potent drugs.

**Keywords:** Bisoprolol, Dimer, Impurities, Synthesis.

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## INTRODUCTION

The purity of a drug product is determined by the ratio of the specified amount of Active Pharmaceutical Ingredient (API) present, as measured by an appropriate analytical method. Even if contaminants do not negatively impact the drug's quality due to their therapeutic effectiveness being equal to or greater than that of the API, the drug substance might still be considered impure if it contains impurities with higher pharmacological or toxicological effects. Regulatory agencies such as the ICH, USFDA, Canadian Drug and Health Agency and others are increasingly focusing on identifying impurities in Active Pharmaceutical Ingredients (APIs) and ensuring that purity standards are met.[1] Synthesis of the material with the proposed structure is a key step in impurity profiling. Developing and validating analytical methods is enhanced by comparing the retention and spectral characteristics of the synthesized material with the impurity of interest. Several recent articles have outlined a methodical approach and guided isolating and identifying process-related impurities and degradation products in pharmaceutical substances using techniques such as mass spectrometry, Nuclear Magnetic Resonance (NMR), High-Performance Liquid Chromatography (HPLC), Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) and Tandem Mass Spectrometry.[2] Reference standards for impurities (related substances) used in drug analysis are readily available on the market, both from pharmacopoeial and non-pharmacopoeial sources. However,

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detailed methods for their synthesis and purification are often lacking. Convenient purification methods for these reference standards offer a cost-effective alternative to the chromatographic techniques mentioned earlier, but they are not widely utilized.[3] Bisoprolol is cardio-selective beta blocker employed in treating heart conditions such as hypertension and coronary heart disease.[4] This beta-adrenergic antagonist decreases the impact of catecholamines (such as adrenaline) by lowering their levels and inhibiting their production. Chemically, it is a derivative of aminopropanol.[5] Bisoprolol is usually administered as a long-term, daily medication. Therefore, it is essential for the product to retain its high quality throughout its shelf life to provide effective and safe treatment for patients.[6]

The synthesis of bisoprolol impurities involves several key steps, including the opening of the epoxide ring, esterification reactions and dimerization reactions. Our objective is to produce derivatives of (Figure 1). Active Pharmaceutical Ingredient (API) impurities and subsequently characterize them using various methods for identification.

## MATERIALS AND METHODS

### Chemicals and solvents

Methanol, Ethyl acetate, Hexane, Dichloromethane, Ethylene glycol monoisopropyl ether, Dimethylformamide, Tetrahydrofuran was purchased from Advent Chembio Pvt. Ltd., Mumbai. *p*-Cresol, Epichlorohydrin, Isopropyl amine, 4-Hydroxy benzoic acid, Thionyl chloride, 4- Hydroxy benzyl alcohol, Acetone were purchased from spectrochem Pvt. Ltd., Mumbai. Potassium carbonate, Sodium sulphate was Merch Specialities Pvt. Ltd, Mumbai. Hydrochloric acid, Sulphuric acid, Potassium hydroxide, Sodium hydroxide was purchased from Fischer Scientific Pvt. Ltd., Mumbai.

### Synthesis of Bisoprolol Impurity R=1-[isopropyl amino]-3-(4-methylphenoxy) propan-2-ol

#### *Step 1: Synthesis of 2-[(p-Tolyoxy) Methyl] Oxirane*

A solution of p-cresol (1.0 eq) was prepared in 40 mL of Dimethylformamide (DMF) under cold conditions and stirred for 5 min. Potassium carbonate ($K_2CO_3$, 1.0 eq) was then added, and stirring continued for 10-15 min. Subsequently, epichlorohydrin (1.5 eq) was added dropwise, and the reaction mixture was heated to 80°C in an oil bath under reflux for 5 hr. Progress was monitored via Thin-Layer Chromatography (TLC) using an n-hexane/ethyl acetate solvent system. Upon completion, the reaction mixture was cooled and poured into ice-cold water to extract DMF. The organic phase was separated using ethyl acetate, and the solution was concentrated under vacuum.

### Step 2: Synthesis of 1-Isopropylamino-3-(4-Methylphenoxy) Propan-2-ol

The crude product from Step 1 (2-[(p-Tolyoxy) Methyl] Oxirane, 1.0 eq) was dissolved in 80-100 mL of methanol under cooled conditions. Isopropylamine (1.5 eq) was then added gradually, and the reaction mixture was stirred for 8-10 min. The mixture was then heated to 80°C in an oil bath and allowed to react for 10-12 hr. Completion of the reaction was confirmed using TLC analysis with a dichloromethane/methanol solvent system. The reaction was quenched with water, and methanol was removed under vacuum. The product was extracted using ethyl acetate and water, followed by concentration to dryness to obtain the final compound. Yield details are presented in Table 1.

### Synthesis of Bisoprolol Impurity K: 2-isopropoxyethyl-4-{2-hydroxy-2-(isopropylmino) propoxy} benzoate

#### *Step 1: Synthesis of 2-Isopropoxyethyl 4-Hydroxybenzoate*

A mixture of 4-hydroxybenzoic acid (1.0 eq) and 30 mL of ethylene glycol mono isopropyl ether was stirred in a Round-Bottom Flask (RBF) for 10 minutes until complete dissolution. The reaction mixture was then cooled to 0-5°C using an ice bath, followed by the slow addition of thionyl chloride (1.2 eq) dropwise, with stirring continuing for 5 min. The reaction mixture was then transferred to an oil bath, heated to 75°C, and refluxed for 10-12 hr. Reaction progress was monitored by Thin-Layer Chromatography (TLC) using n-hexane/ethyl acetate as the mobile phase. Upon completion, the reaction mixture was processed using ethyl acetate and water, and the organic and aqueous layers were separated. The organic layer was then concentrated.

### Step 2: Synthesis of 2-Isopropoxyethyl 4-[Oxiran-2-ylmethoxy] Benzoate

The crude product from Step 1 (1.0 eq) was dissolved in 50 mL of Dimethylformamide (DMF). Potassium carbonate ($K_2CO_3$, 1.0 eq) was added, and the reaction mixture was stirred for 10 minutes at 0-5°C. Epichlorohydrin (1.2 eq) was then added dropwise, and the temperature was gradually increased to 80°C. The mixture was refluxed for 7-8 hr, and reaction completion was confirmed using TLC with n-hexane/ethyl acetate as the mobile phase. After completion, the reaction was quenched by adding water, followed by extraction using ethyl acetate and water. The layers were separated, and the organic layer was concentrated.

### Step 3: Synthesis of 2-Isopropoxyethyl 4-[2-Hydroxy-3-(Isopropylamino) Propoxy] Benzoate

The product from Step 2 (1.0 eq) was dissolved in 50 mL of methanol and cooled to 0-5°C. Isopropylamine (1.5 eq) was added dropwise, and the reaction mixture was stirred. The temperature was then increased to 80°C, and the mixture was

refluxed for 8-10 hr. Reaction completion was assessed using TLC with dichloromethane/methanol as the mobile phase. Upon completion, the reaction was neutralized with water, followed by extraction using ethyl acetate and water. The layers were separated, and the organic layer was fully concentrated. Percentage yield details are provided in Table 1.

### Synthesis of Bisoprolol Impurities and Dimer Impurity

#### *Step 1: Preparation of Activated Silica*

A reaction flask was charged with silica (20 g) and acetone (60 mL) at 25°C. The mixture was stirred, followed by the addition of concentrated sulfuric acid (13 mL). The reaction mass was stirred for 1 hr, and the solvent was then removed under vacuum to obtain activated silica.

### Step 2: Synthesis of 4-{(2- Isopropoxyethoxy) Methyl} Phenol (Bisoprolol impurity M)

A round-bottom flask (RBF) was charged with 2-isopropoxyethanol (12.5 mL) and stirred in an ice bath for 5 minutes. Sulfuric acid-absorbed activated silica (1.0 eq) was then introduced, followed by the addition of 4-hydroxybenzyl alcohol (1.0 eq). The reaction mixture was stirred for 5 min, after which cooling was removed, and the reaction was allowed to proceed at Room Temperature (RT) for 5-6 hr. Progress was monitored by Thin-Layer Chromatography (TLC) using n-hexane/ethyl acetate as the mobile phase. Upon completion, the reaction mixture was filtered through a Büchner funnel to recover silica. The filtrate was treated with potassium carbonate (0.5 eq) and stirred for 1 hour, followed by another filtration to remove excess $K_2CO_3$. The reaction mixture was then extracted using toluene and water, and the organic layer was concentrated under vacuum.

### Step 3: Synthesis of Intermediate 1: 2-(4-(2-Isopropoxyethoxymethyl) Phenoxymethyl) Oxirane

The product from Step 2 (1.0 eq) was dissolved in 100 mL of Dimethylformamide (DMF) under cooling conditions and stirred for 5 min. Potassium carbonate (1.0 eq) was then added, and stirring continued for 15 min. Epichlorohydrin (1.2 eq) was added dropwise, and after completion, the cooling was discontinued. The reaction mixture was transferred to an oil bath and refluxed at 80°C for 6 hr. Reaction completion was confirmed using TLC with n-hexane/ethyl acetate as the mobile phase. The reaction mixture was then cooled, transferred into ice-cold water to remove DMF, and extracted using ethyl acetate. The final solution was concentrated under vacuum.

### Synthesis of Bisoprolol Impurity J: 3-{4-((2-Isopropoxyethoxy) Methyl) Phenoxy}-1,2-Propanediol

The intermediate from Step 3 (1.0 eq) was dissolved in Tetrahydrofuran (THF, 25 mL), and distilled water (10 mL) was added under cooling conditions. After stirring for 10-15 min, potassium hydroxide (10 drops) was introduced. Cooling was then discontinued, and the reaction mixture was transferred to an oil bath, maintained at 60°C for 4 hr. Completion was verified by TLC using dichloromethane/methanol as the mobile phase. The reaction mixture was extracted with ethyl acetate and water, followed by rotary evaporation to concentrate the organic layer.

### Synthesis of Bisoprolol Dimer Impurity: 3,3′-(Isopropylazanediyl) Bis [1-(4-((2-Isopropoxyethoxy) Methyl) Phenoxy) Propan-2-ol]

The intermediate from Step 3 (1.0 eq) was dissolved in 30 mL of methanol under cooling conditions. The mixture was stirred for 10-15 min, followed by the addition of isopropylamine (0.5 eq). Cooling was then discontinued, and the reaction mixture was transferred to an oil bath, maintained at 80°C for 3-4 hr. Completion was confirmed using TLC with dichloromethane/methanol as the mobile phase. The reaction mixture was extracted with ethyl acetate and water, and the organic layer was fully concentrated using a rotary evaporator. Percentage yield details are provided in Table 1.

### Purification of Synthesized Compounds by Column Chromatography

Column chromatography stands as a fundamental method in the realm of chemical separation and purification. The technique involves the passage of a liquid mobile phase through a column filled with a solid stationary phase. As the mixture descends through the column, compounds separate from each other, driven by diverse interactions with the stationary phase. Column chromatography plays a pivotal role in the isolation and purification of chemicals and finds widespread application in both research and industrial settings.

## METHOD OF ANALYSIS

### Thin Layer Chromatography (TLC)

For TLC analysis, an aluminum sheet pre-coated with silica gel was employed. The spots on the TLC plates were visualized under UV light.

### Infrared Spectroscopy

Infrared spectra of the synthesized compounds were recorded using a JASCO FTIR-4100 spectrophotometer, with KBr pellets used as the sample medium. The spectra were expressed in wavenumbers ($cm^{-1}$).

### Mass Spectrometry

Mass spectra were obtained using a Bruker Impact HD mass spectrometer.

### Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were acquired with a MERCURY VARIAN 500 MHz or 400 MHz instrument, using $CDCl_3$ or DMSO as solvents (unless otherwise noted) and Tetramethylsilane (TMS) as the internal standard. Chemical shifts are reported in δ (ppm).

## RESULTS

The synthesis of Bisoprolol Impurity R, Bisoprolol Impurity K, Bisoprolol Impurity M, Bisoprolol Impurity J and Bisoprolol Dimer Impurity was carried out in compliance with the suggested route in Figures 2-4 respectively. Purification of each step was done by column chromatography. $^1$H NMR and Mass data of all synthesized impurities satisfied the structure elucidation requirements, Bisoprolol Impurity R, having the molecular formula $C_{13}H_{21}NO_2$ shows a mass m+1 peak at 224.2 and NMR satisfied all the 21 protons present in the structure. Bisoprolol Impurity K, having molecular formula $C_{18}H_{29}NO_5$ shows a mass m+1 peak at 340.2 and proton NMR proves the presence of total 29 protons in spectral data, Bisoprolol Impurity M, $C_{12}H_{18}O_3$ shows a M peak at 210.2 which matches the molecular weight of said impurity and NMR spectra proves the presence of 18 protons in its structure. Bisoprolol Impurity J, having molecular formula $C_{15}H_{24}O_5$ mass data shown M-1 peak at 283.2 and NMR data shows a presence of 24 protons in its structure, Bisoprolol Dimer Impurity $C_{33}H_{53}NO_8$ shows an M+1 peak at 592.4 and satisfied the NMR data for 51 protons.

## DISCUSSION

In this research paper, a total of five impurities of Bisoprolol were synthesized. The methods and procedures developed for this synthesis are entirely novel and straightforward, making them suitable for small-scale synthesis. These methods will be valuable for undergraduate and postgraduate students, as well as researchers, in synthesizing these impurities in laboratory settings. The IR spectroscopy results were outstanding and the mass spectra and NMR data showed strong alignment with the structural requirements of all the synthesized impurities.

### Characterization of Compounds

#### *Spectral Data of Bisoprolol Impurity R: 1-(isopropylamino)-3-(4-methylphenoxy) propan-2-ol*

The solid-state IR spectrum of this compound (KBr, $cm^{-1}$) displays an O-H stretching band at 3306.36 $cm^{-1}$, a moderate N-H stretching peak at 3025.36 $cm^{-1}$ and a prominent C-O stretching absorption at 1337.39 $cm^{-1}$. Additionally, there is a strong C-H bending feature at 1089.58 $cm^{-1}$. The $^1$H NMR spectrum (400 MHz, $CDCl_3$) shows the following signals: 1.082-1.10 ppm (doublet, 6H, $CH_3$), 2.28 ppm (singlet, 3H, $CH_3$), 2.70-2.75 ppm (multiplet, 1H, CH), 2.81-2.91 ppm (multiplet, 2H, $CH_2$), 3.93-3.95 ppm (doublet, 2H, $CH_2$), 4.00-4.03 ppm (multiplet, 1H, CH), 2.41-2.43 ppm (broad singlet, 2H, OH, NH), 7.062-7.08 ppm (doublet, 2H, CH) and 6.97-6.83 ppm (doublet, 2H, CH). The mass spectrometry data for $C_{13}H_{21}NO_2$ show a calculated molecular weight of 223 g/mol, with experimental mass-to-charge ratios of 223.3 ($M^+$) and 224.2 ($M^+H$).

#### Spectral Data of Bisoprolol Impurity K: 2-isopropoxyethyl-4-(2-Hydroxy-3-(isopropylamino) propoxy) benzoate

In the liquid-state IR spectrum (KBr, $cm^{-1}$), this chemical features an O-H stretch at 3419.60 $cm^{-1}$, a moderate N-H stretch at 3042.16 $cm^{-1}$, a strong C=O stretching absorption at 1709.23 $cm^{-1}$, a strong C-O stretch at 1291.69 $cm^{-1}$ and a prominent C-H bending peak at 747.52 $cm^{-1}$. The $^1$H NMR spectrum (400 MHz, $CDCl_3$) exhibits the following peaks: 7.98-8.00 ppm (doublet, 2H, CH), 6.91-6.93 ppm (doublet, 2H, CH), 4.39-4.41 ppm (triplet, 2H, $CH_2$), 1.11-1.13 ppm (doublet, 6H, $CH_3$), 1.17-1.18 ppm (doublet, 6H, $CH_3$), 4.05-4.09 ppm (multiplet, 1H, CH), 3.62-3.68 ppm (multiplet, 1H, CH), 3.72-3.75 ppm (triplet, 2H, $CH_2$) and 4.00-4.03 ppm (doublet, 2H, $CH_2$). The mass spectrometry data for $C_{18}H_{29}NO_5$ indicate a calculated molecular weight of 339 g/mol, with experimental mass-to-charge ratios of 339.4 ($M^+$) and 340.2 ($M^+H$).

#### Spectral Data of Bisoprolol Impurity M: 4-[(2-isopropoxyethoxy) methyl] phenol

The liquid-state IR spectrum ($cm^{-1}$) reveals an O-H stretch at 3360.91 $cm^{-1}$, a strong C-O stretching band at 1333.25 $cm^{-1}$ and a significant C-H bending absorption at 767.50 $cm^{-1}$. In the $^1$H NMR spectrum (400 MHz, $CDCl_3$), the signals are: 1.16-1.18 ppm (doublet, 6H, $CH_3$), 3.58-3.65 ppm (multiplet, 5H, O-$CH_2$, O-CH), 4.5 ppm (singlet, 2H, $CH_2$), 7.13-7.15 ppm (doublet, 2H,

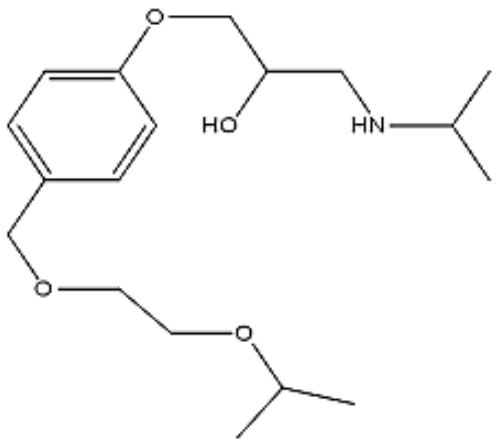



**Figure 1:** Lead API use for Impurity Synthesis (Bisoprolol).

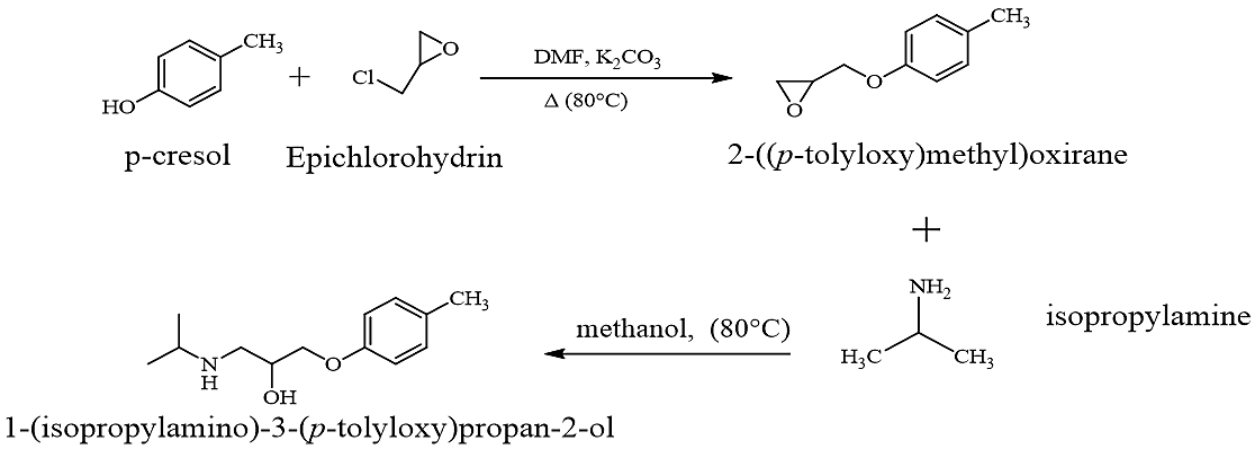



**Figure 2:** The Scheme for Synthesis of Bisoprolol Impurity R.

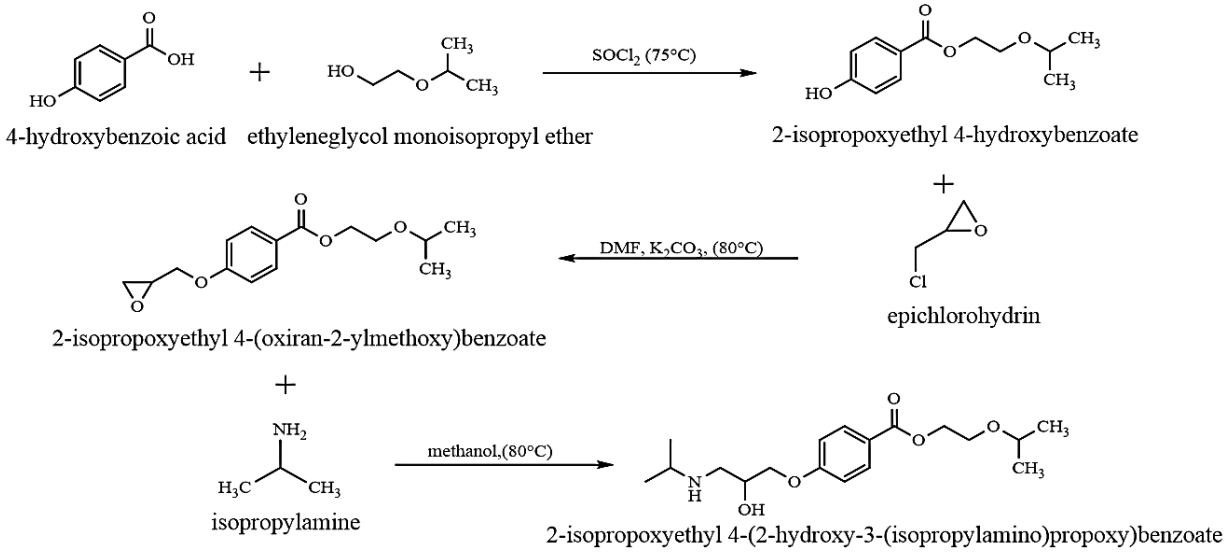



**Figure 3:** The Scheme for Synthesis of Bisoprolol Impurity K.

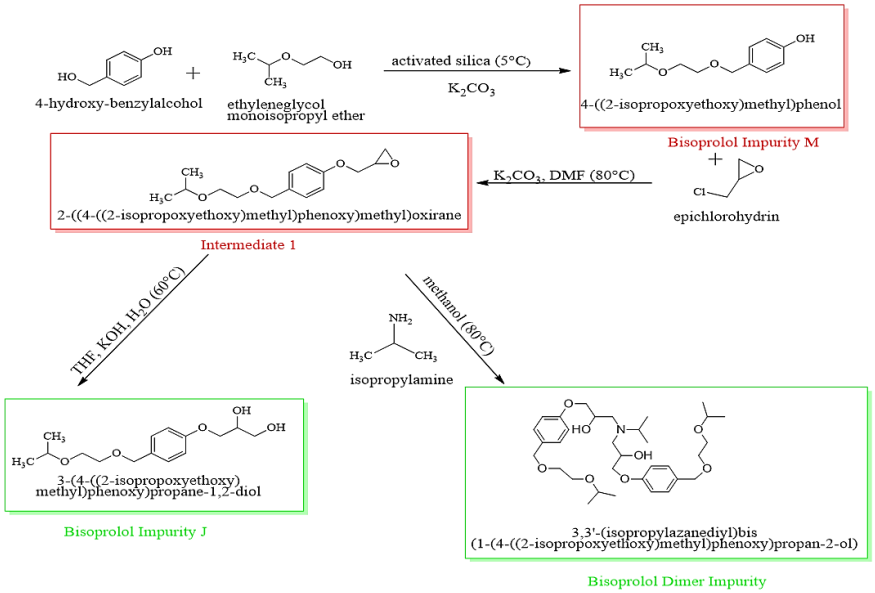
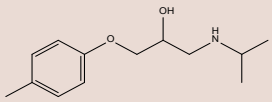
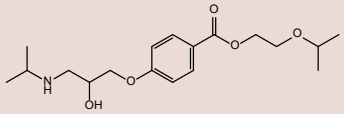
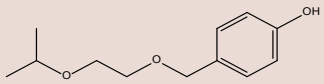
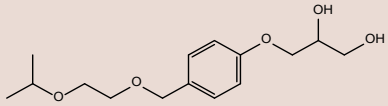
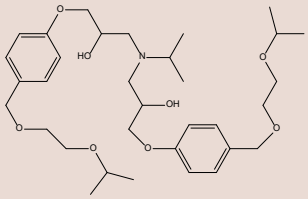



**Figure 4:** The Scheme shows the synthetic route for Bisoprolol Impurity M, J and Dimer.

**Table 1: Properties of Synthesized Impurities of Bisoprolol.**

| Sl. No. | Name of Impurity | Structure and IUPAC Name | Molecular formula | Molecular weight | Appearance | Yield (%) |
|---|---|---|---|---|---|---|
| 1 | Bisoprolol Impurity R |  1-(isopropylamino)-3-[4-methylphenoxy] propan-2-ol. | $C_{13}H_{21}NO_2$ | 223g $mol^{-1}$ | White solid | 58.30 |
| 2 | Bisoprolol Impurity K |  2-isopropoxyethyl-4-(2-hydroxy-3-[isopropylamino] propoxy) benzoate. | $C_{18}H_{29}NO_5$ | 339 g $mol^{-1}$ | Yellow liquid | 21.90 |
| 3 | Bisoprolol Impurity M |  4-{(2-isopropoxyethoxy)methyl} phenol. | $C_{12}H_{18}O_3$ | 210 g $mol^{-1}$ | Yellow liquid | 42.83 |
| 4 | Bisoprolol Impurity J |  3-[4-{(2-isopropoxyethoxy)methyl} phenoxy]-1,2-propanediol. | $C_{15}H_{24}O_5$ | 284 g $mol^{-1}$ | Yellow liquid | 30.25 |
| 5 | Bisoprolol Dimer Impurity |  3,3'-[isopropylazanediyl] bis {1-(4-((2-isopropoxyethoxy) methyl) phenoxy} propan-2-ol. | $C_{33}H_{53}NO_8$ | 591 g $mol^{-1}$ | Yellow liquid | 21.34 |

CH), 6.71-6.73 ppm (doublet, 2H, CH) and 6.33 ppm (singlet, 1H, OH). The mass spectrometry data for $C_{12}H_{18}O_3$ show a calculated molecular weight of 210.3 g/mol, with experimental mass-to-charge ratios of 210.3 ($M^+$) and 210.2 ($M^+$).

## Spectral Data of Bisoprolol Impurity J: 3-[4-((2-isopropoxyethoxy) methyl) phenoxy]-1, 2-propanediol

The liquid-state IR spectrum ($cm^{-1}$) displays an O-H stretch at 3416.92 $cm^{-1}$, a strong C-O stretch at 1250.83 $cm^{-1}$ and a strong C-H bending peak at 796.21 $cm^{-1}$. The $^1H$ NMR spectrum (400 MHz, $CDCl_3$) shows: 7.27-7.28 ppm (doublet, 2H, $CH_2$), 6.86-6.88 ppm (doublet, 2H, $CH_2$), 4.50 ppm (singlet, 2H, $CH_2$), 1.15-1.17 ppm (doublet, 6H, $CH_3$), 2.185 ppm (broad singlet, 2H, OH), 4.06-4.09 ppm (multiplet, 1H, CH), 4.01-4.02 ppm (doublet, 2H, O-$CH_2$), 3.58-3.65 ppm (multiplet, 5H, O-$CH_2$, O-CH) and 3.72-3.84 ppm (doublet of doublets, 2H, $CH_2$). The mass spectrometry data for $C_{15}H_{24}O_5$ indicate a calculated molecular weight of 284.4 g/mol, with experimental mass-to-charge ratios of 284.4 ($M^+$) and 283.2 (M-H).

## Spectral Data of Bisoprolol Dimer Impurity: 3, 3' -(isopropylazanediyl) bis 1-(4-((2-isopropoxyethoxy) methyl) phenoxy) propan-2-ol

The liquid-state IR spectrum ($cm^{-1}$) features an O-H stretch at 3271.42 $cm^{-1}$, a moderate N-H stretch at 2944.65 $cm^{-1}$, a strong C-O stretching band at 1247.56 $cm^{-1}$ and a prominent C-H bending peak at 747.97 $cm^{-1}$. The $^1H$ NMR spectrum (400 MHz, $CDCl_3$) includes: 7.25-7.27 ppm (doublet, 4H, CH), 6.86-6.89 ppm (multiplet, 4H, CH), 4.5 ppm (singlet, 4H, $CH_2$), 3.57 ppm (multiplet, 6H, $CH_2$-O), 3.58-3.62 ppm (multiplet, 2H, OH), 3.96-3.98 ppm (doublet, 4H, CH), 4.05-4.07 ppm (multiplet, 2H, O-$CH_2$), 2.61-2.82 ppm (multiplet, 4H, O-$CH_2$, O-CH), 3.05-3.08 ppm (multiplet, 1H, $CH_2$), 1.03-1.13 ppm (doublet, 6H, $CH_3$) and 1.16-1.17 ppm (doublet, 12H, $CH_3$). The mass spectrometry data for $C_{33}H_{53}NO_8$ reveal a calculated molecular weight of 591 g/mol,

with experimental mass-to-charge ratios of 591 ($M^+$) and 592.4 ($M^+H$).

*All NMR and Mass spectra of synthesized compounds are attached with supplementary datafile.

## CONCLUSION

The synthesis of Bisoprolol Impurity R began with the synthesis of intermediate 2-((p-tolyloxy) methyl) oxirane from p-cresol and epichlorohydrin. When this intermediate was treated with isopropyl amine, it underwent an epoxide ring-opening reaction, resulting in the formation of 1-(isopropyl amino)-3-(p-tolyloxy) propan-2-ol. The purification of these compounds was achieved through column chromatography, yielding a final product with a percentage yield of 58.30%. The synthesis of impurity K involved a series of chemical reactions. It began with the reaction of 4-hydroxybenzoic acid and ethylene glycol monoisopropyl ether through an esterification reaction. This reaction resulted in the formation of an intermediate known as 2-isopropoxyethyl 4-hydroxybenzoate. Subsequently, this intermediate underwent a reaction with epichlorohydrin, leading to the formation of a second intermediate called 2-isopropoxyethyl 4-(oxiran-2-ylmethoxy) benzoate. The second intermediate then underwent an epoxide ring opening reaction, using isopropyl amine as a weak base. This reaction resulted in the formation of 2-isopropoxyethyl 4-(2-hydroxy-3-(isopropyl amino) propoxy) benzoate. The overall process yielded this final product with a percentage yield of 21.90%. The synthesis of Bisoprolol Impurity M began with the preparation of activated silica. Subsequently, 4-hydroxy-benzyl alcohol, serving as the starting material, underwent a reaction with ethylene glycol monoisopropyl ether, resulting in the formation of the product known as 4-((2-isopropoxyethoxy) methyl) phenol. Bisoprolol Impurity J and the Bisoprolol Dimer Impurity were synthesized in a multi-step process, starting with the synthesis of Bisoprolol Impurity M. In the initial step, Bisoprolol Impurity M was produced. This intermediate, referred to as Intermediate 1, then underwent a reaction with epichlorohydrin to generate Intermediate 2. Subsequently, Intermediate 2 was subjected to further reactions with KOH and isopropyl amine, resulting in the formation of Bisoprolol Impurity J and Bisoprolol Dimer Impurity. The yields obtained for these products were 30.25% and 21.34%, respectively. These impurities were characterized using analytical techniques such as IR, $^1H$ NMR, and Mass Spectrometry.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**ICH:** International Council for Harmonisation; **USFDA:** United State Food and Drug Administration; **API:** Active Pharmaceutical Ingredient; **RBF:** Round Bottom Flask; **DMF:** Dimethylformamide; **RT:** Room Temperature; **TLC:** Thin Layer Chromatography; **KOH:** Potassium hydroxide; **THF:** Tetrahydrofuran; **NMR:** Nuclear Magnetic Resonance; **IR:** Infrared; **HPLC:** High-Performance Liquid Chromatography; **FTICR-MS:** Fourier Transform Ion Cyclotron Resonance Mass Spectrometry.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study not involve animal or human participants for any trial so that no need of Ethics Approval and Consent to Participate.

## SUMMARY

In this research paper, we explore novel methods for synthesizing five Bisoprolol impurities: Bisoprolol Impurity-R, Bisoprolol Impurity-K, Bisoprolol Impurity-M, Bisoprolol Impurity-J and the Bisoprolol Dimer Impurity. The spectral data confirm the successful synthesis of these impurities, showing excellent agreement with their respective structures. These methods are simple, robust and will facilitate the synthesis of these impurities in laboratories with good yield.

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