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Original Article

Synthesis, Purification and Characterization of Some Impurities of Propranolol

Pradnya Sangram Waghmare[1], Amit Suryakant Tapkir[1,*], Pavin Digambar Chaudhari[3], Ashok Bhau Pathak[2], Sandip Murlidhar Bhagat[2], Sneha Nandkumar Rashinkar[1], Anuradha Ghanshyam More[3], Padmaja Santosh Kore[4]

[1]Department of Pharmaceutical Chemistry, PES's, Modern College of Pharmacy, Nigdi, Pune, Maharashtra, INDIA.
[2]Department of Synthetic Chemistry, Anasyn Lab Private Limited, Tathawade, Pune, Maharashtra, INDIA.
[3]Department of Pharmaceutics, PES's, Modern College of Pharmacy, Nigdi, Pune, Maharashtra, INDIA.
[4]Department of Pharmacology, PES's, Modern College of Pharmacy, Nigdi, Pune, Maharashtra, INDIA.

ABSTRACT

Introduction: During the process of drug development, control of impurities and it should keep within the prescribed limit is very important to get high-qualified drugs. Several studies have been dedicated to synthesizing the impurities and studying the structures to support the method of purification. Propranolol is beta blocker medication preferably blocks β1 receptor, primarily used to treat high blood pressure and heart-associated chest pain. Hence synthesis and study of impurities related to propranolol is very important variables. **Aim:** The aim of this research work has to synthesize propranolol impurities because these impurities play a vital role in the drug process. **Experimental work:** We focus on synthesis of five main propranolol impurities with purification and characterization. We synthesize five impurities namely: 2-[(naphthalen-1-yloxy) methyl] oxirane; 3,3'-(isopropyl azanediyl) bis(1-(naphthalen-1-yloxy) propan-2-ol) hydrochloride; 1,3-bis(naphthalen-1-yloxy) propan-2-ol; 1-amino-3-naphthalen-1-yloxypropan-2-ol; and N-(2-Hydroxy-3-(naphthalen-1-yloxy) propyl)-N-isopropyl acetamide. These five impurities were designed and synthesize by ring opening, dimerization and reduction by taking 1 naphthol as starting material. **Characterization:** Column chromatography and thin-layer chromatography techniques were used for the separation and purification of chemical compounds. The structures of synthesized compounds were elucidated by using mass spectrometry and ^{1}H-NMR spectroscopy. For future prospect pharmaceutical industry reduce the level of impurity to their required threshold according to ICH guideline. **Conclusion:** We synthesized impurity which belong to emergency cardiovascular drug, so that they have high market value in pharmaceutical industry for concerning safety of potent drug. In our research work, we study how to synthesize impurities of API and their characterization.

Keywords: Propranolol, Impurity Synthesis, 2-[(naphthalen-1-yloxy) methyl] oxirane, Ring opening, Dimerization.

Correspondence:
Dr. Amit Suryakant Tapkir
Associate Professor, Department of Pharmaceutical Chemistry, PES, Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, INDIA.
Email: amittapkir.8@gmail.com

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INTRODUCTION

One of the original beta-blocker drugs, propranolol (Figure 1a) is used for the treatment of cardiovascular diseases, tremors, angina, and hypertension.[1,2]

Along with treating anxiety-related conditions, infantile hemangiomas, and headaches caused by migraines, propranolol is also an effective and safe medication.[3,4] Impurity synthesis of propranolol involves producing impurities, which may be generated during the synthesis process as well as present in the end product. Additionally, a number of recent studies show that individuals using propranolol have a lower chance of developing stomach, colon and prostate cancers.[5-7] Propranolol's impurity synthesis is useful for method development, validation, stability studies, quality assurance, and toxicological analysis.[8] In a quick, low-toxicity reaction process, propranolol was produced by reacting 1-naphthol with isopropyl amine.[9] Epoxide ring opening reactions, Fischer esterification reactions, and dimer reactions are the primary steps in the synthesis of propranolol impurities. Our aim is to synthesize the impurity of API derivatives and their characterization by using different methods for impurity identification. Propranolol impurity EP A, propranolol impurity B, propranolol impurity C, and propranolol impurity N Desisopropyl are synthesized (for details refer to Table 1) with good yields using the common intermediate 2-[(naphthalen-1-yloxy) methyl] oxirane (Figure 1b) and propranolol impurity 2 synthesized from Propranolol API.

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Experimental Work

Chemical material

Methanol, ethyl acetate, hexane, dichloromethane, epichlorohydrin, potassium carbonate, ethanol, formic acid, iodine, Isopropyl amine, Sodium thiosulphate were purchased from Advent Chembio Pvt. Ltd, Mumbai. Methanol (anhydrous), 1- naphthol, epichlorohydrin, Acetone, Phenol, DMF, Sodium thiosulphate, Ammonia hydroxide were purchased from Spectrachem Pvt. Ltd., Mumbai. NaOH, HCl, Sulphuric acid, THF, Acetyl chloride were purchased from Fischer Scientific Pvt. Ltd, Mumbai.

Synthesis of Propranolol Impurities

During the synthesis, some impurities are produced that have a higher yield than the API. As a result, we produce impurities A, B, C, desisopropyl from the intermediate (Figure 1b) and impurity 2 from propranolol. For the synthesis of a propranolol impurity, the epoxide ring opening reaction is commonly used. At times, a dimerization reaction takes place during the synthesis of impurities B and C (Figure 2).

Synthesis of 2-[(naphthalen-1-yloxy) methyl] oxirane (Intermediate)

Take one equivalent of naphthalen-1-ol (10 g), 1.5 equivalents of epichlorohydrin, and one equivalent of a base (Potassium carbonate). Weigh out 10 g of naphthalen-1-ol and dissolve it in 100 mL of DMF solvent. Add the base to the naphthalen-1-ol under continuous stirring at cold conditions. After 10 min, slowly add epichlorohydrin dropwise to the mixture while stirring, and maintain the reaction overnight at 80°C. Once the reaction completion is confirmed by TLC, proceed with the workup. Quench the reaction with ice and ethyl acetate, then separate the organic and aqueous layers. Eliminate any remaining water from the organic layer by introducing anhydrous sodium sulfate. Concentrate the organic layer, dry it, and obtain the 2-[(naphthalen-1-yloxy) methyl] oxirane as an intermediate.

Synthesis of Impurity A from intermediate 2-[(naphthalen-1-yloxy) methyl] oxirane

Begin by taking an equivalent of 0.5 g of the intermediate 2-[(naphthalen-1-yloxy) methyl] oxirane. Add 2 mL of THF to the intermediate, followed by the addition of 2 mL of water to facilitate dissolution. Apply cooling for 10 min. Next, add 2 to 3 drops of sulfuric acid dropwise and stir the mixture for 30 min. Then, place the reaction mixture under reflux with continuous heating at 60-70°C for 8 hr. Once the reaction is complete, concentrate the reaction mass. Separate the organic layer from the mixture using ethyl acetate and water. Remove the water content from the organic layer by introducing anhydrous sodium sulfate. Concentrate the organic layer, filter it, and dry it to obtain 3-(naphthalen-1-yloxy) propane-1,2-diol.

Synthesis of Impurity B from intermediate 2-[(naphthalen-1-yloxy) methyl] oxirane

Dissolve 2-((naphthalen-1-yloxy) methyl) oxirane in 20 mL of methanol with stirring. Add propylamine to the solution, maintaining stirring. Slowly add methanol dropwise to the mixture while maintaining cold conditions and stirring. Place the reaction mixture under reflux conditions for 8 hr to allow the reaction to proceed. Once the reaction is complete, concentrate the reaction mixture. Separate the organic layer from the mixture by using ethyl acetate and water. Remove water from the organic layer by adding anhydrous sodium sulfate. Concentrate the organic layer and then filter it. Dry the obtained material to yield the 3,3'-(isopropyl azanediyl) bis(1-(naphthalen-1-yloxy) propan-2-ol) hydrochloride.

Synthesis of Impurity C from intermediate 2-[(naphthalen-1-yloxy) methyl] oxirane

Start by taking 1 equivalent of the intermediate 2-((naphthalen-1-yloxy) methyl) oxirane, along with 1 equivalent of naphthalen-1-ol and 1 equivalent of NaOH. Prepare a NaOH solution by dissolving NaOH flakes in 2-3 mL of water. Dissolve 2-((naphthalen-1-yloxy) methyl) oxirane in 20 mL of ethanol with stirring. Then, add naphthalen-1-ol and slowly introduce NaOH dropwise while maintaining cold conditions and stirring. Allow the mixture to undergo reflux for 8 hr. Upon completion of the reaction, concentrate the reaction mass. Separate the organic layer from the mixture using a combination of ethyl acetate and water. To remove water from the organic layer, introduce anhydrous sodium sulfate. Concentrate the organic layer, filter it, and dry to yield 1,3-bis(naphthalen-1-yloxy) propan-2-ol.

Synthesis of Impurity N-Desis propyl from intermediate 2-[(naphthalen-1-yloxy) methyl] oxirane

Begin by taking 1 equivalent of 3 g of the intermediate 2-((naphthalen-1-yloxy) methyl) oxirane. Add 20 mL of THF and then introduce 10 mL of ammonia hydroxide. Stir the reaction mixture and place it under reflux with continuous heating at 40°C overnight. Monitor the progress of the reaction using TLC (thin-layer chromatography). After the reaction is complete, concentrate the reaction mass. Separate the organic layer from the mixture using a combination of ethyl acetate and water. Remove water from the organic layer by adding anhydrous sodium sulfate. Concentrate the organic layer, filter it, and dry it to obtain 1-amino-3-naphthalen-1-yloxypropan-2-ol.

Synthesis of Impurity 2 from intermediate 2-[(naphthalen-1-yloxy) methyl] oxirane via Propranolol

Step I: Synthesis of propranolol from Intermediate

The intermediate, dissolved in methanol, was added to isopropyl amine and stirred at 45°C until a yellow-brown solution was obtained (48 hr). The solution was then cooled to 5°C. A slow and gradual addition of 2M HCl was performed into the resulting mixture, followed by the addition of 2M NaOH until a white precipitate appeared.

Step II: Synthesis of Impurity 2 from Propranolol

Begin by taking 1 equivalent of propranolol API (2 g), 1 equivalent of acetyl chloride, and 1 equivalent of triethylamine. Dissolve 2 g of propranolol API in Dichloromethane (DCM) while maintaining cooling, and stir the mixture for 30 min. Then, add 1 mL of triethylamine dropwise, followed by the gradual addition of 0.5 mL of acetyl chloride dropwise while stirring. Reflux the reaction mixture for 8 hr with continuous heating at a temperature of 60-70°C. Monitor the progress of the reaction using TLC. Once the reaction is complete, as indicated by TLC, proceed with the workup. Quench the reaction by adding ice and ethyl acetate. Separate the resulting organic and aqueous layers. Eliminate any remaining water from the organic layer by introducing anhydrous sodium sulfate. Concentrate the organic layer and then dry it to obtain N-(2-Hydroxy-3-(naphthalen-1-yloxy) propyl)-N-isopropyl formamide.

Method of Purification

In chemical separation and purification, column chromatography is a fundamental technique. In this method, a liquid mobile phase is passed into a column that has been filled with a solid stationary phase. Compounds separate from one another as the mixture lowers into the column due to different interactions between them and the stationary phase. Column chromatography is essential for isolating and purifying chemicals and is commonly used in both research and industry.

Method of Characterization

Thin layer Chromatography

A quick and affordable chromatographic method used in a variety of research fields is TLC. TLC provides rapid qualitative analysis by separating chemicals in a mixture according to their different affinities for stationary and mobile phases. In TLC, a small amount is observed on a plate coated in an absorbing material substance. The plate is then put in a chamber that is filled with solvent, where capillary action allows the solvent to flow over it and separate the parts of the mixture into various parts. Researchers now have a useful tool for effective analysis and characterization thanks to this approach, which is useful for determining reaction progress, chemical purity, and more.

Mass Spectroscopy

Mass spectrometry is a powerful analytical technique used to identify and quantify the mass-to-charge ratio of ions in a sample. It helps in understanding the chemical composition and structure of a wide range of items, from small molecules to big macromolecules. Mass spectrometry produces ions by introducing a sample to high-energy electrons or various ionization techniques; these ions are then separated in a magnetic field according to their mass-to-charge ratios. This separation allows for accurate determination of a compound's molecular weight and structural information, aiding in applications like identifying unknown compounds, studying molecular interactions, and analyzing complex mixtures.

Nuclear Magnetic Resonance

Nuclear Magnetic Resonance (NMR) spectroscopy is a non-destructive analytical technique used to study the molecular structure, dynamics, and interactions of organic compounds. It relies on the interaction between nuclear spins and an external magnetic field, producing distinct signals that reveal information about chemical environments and the connectivity of atoms within a molecule. By applying radiofrequency pulses, NMR spectroscopy generates spectra that display resonances corresponding to different nuclei, allowing researchers to deduce valuable insights into molecular conformation, functional groups, and intermolecular associations. It finds applications in diverse fields, including chemistry, biochemistry, and drug discovery.

RESULTS

The summary of the research work is to synthesize impurities of propranolol API, which are Propranolol impurities A, B, C, 2, and N Desisopropyl impurity. 1 naphthol is the starting material for the synthesis of 5 impurities of propranolol API. The basic ring opening reaction is used for the synthesis of an intermediate of five impurities. 2-[(naphthalen-1-yloxy) methyl] oxirane is an intermediate is used for the synthesis of impurities A, B, C, 2 and N Desisopropyl impurity. Synthesis of Impurity A by adding intermediate to sulphuric acid for ring opening and THF to produce impurity A with 83% yield and a white appearance. Synthesis of Impurity B is dimerization with intermediate in the presence of isopropylamine to get 74% yield in white solid. Synthesis of impurity c by adding 1 naphthol to intermediate in presence of THf and aq. NaOH to give 66.7% yield in brown sticky appearance. Synthesis of N Desisopropyl impurity to add ammonia to the intermediate to produce a white solid with 72.61% yield of impurity. Synthesis of impurity 2 from propranolol by adding acetyl chloride and THf to produce a light brown colour of impurity 1 with 75.2% yield. All five impurities are purified by column chromatography.

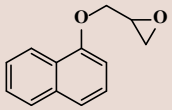
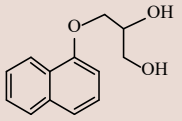
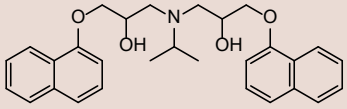
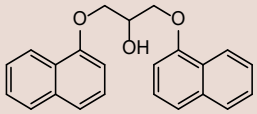
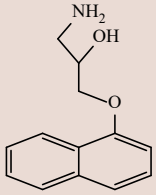
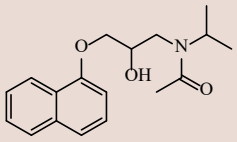
^{1}H NMR and Mass data of all synthesized impurities satisfied the structure elucidation requirements, impurity A having the

molecular formula $C_{13}H_{14}O_3$ shows a mass m+1 peak at 201 and NMR satisfied all the 14 protons present in the structure Impurity B, having molecular formula $C_{29}H_{33}NO_4$ shows a mass m+1 peak at 460.2 and proton NMR proves the presence of total 33 protons In spectral data, impurity C shows a M+1 peak at 345.17 which matches the mole cular weight of said impurity and NMR spectra proves the presence of 20 protons in its structure Impurity 2 mass data shown M+1 peak at 302.2 and NMR data shows a presence of 23 protons in its structure (, N Desisopropyl Impurity shows an M+1peak at 218.4 and satisfied the NMR data for 15 protons

DISCUSSION

In this research paper total five impurities of propranolol were synthesized by using propranolol as the starting material. The method and procedure used for this impurity synthesis are totally novel and simple for small-scale synthesis. This will help undergraduate and postgraduate students, as well as researchers to synthesize these impurities in their laboratories. The IR spectroscopy shows very excellent results, and the mass spectra and NMR data show very good agreement with the structural requirements of all synthesized impurities.

Table 1: Characteristics of Synthesized Impurities of Propranolol.

Sl. No.	Name of Impurity	Structure And IUPAC	Appearance	Yield (%)	Melting Point (°C)
1	Intermediate	 2-[(naphthalen-1-yloxy) methyl] oxirane	Off White Solid	60.59%	192-194°C
2	Impurity A	 2-[(naphthalen-1-yloxy) methyl] oxirane	Off White Solid	79.10%	94-96°C
3	Impurity B	 3,3'-(isopropyl azanediyl) bis(1-(naphthalen-1-yloxy) propan-2-ol) hydrochloride	Off White Solid	73.68%	105-107°C
4	Impurity C	 1,3-bis(naphthalen-1-yloxy) propan-2-ol	Brown Sticky Solid	66.76%	162-164°C
5	N -deisopropyl Impurity	 1-amino-3-naphthalen-1-yloxypropan-2-ol	White to Off-White Solid	72.61%	166-168 °C
6	Impurity 2	 N-(2-Hydroxy-3-(naphthalen-1-yloxy) propyl)-N-isopropyl acetamide	Light Brown Solid	76.29%	134-136 °C

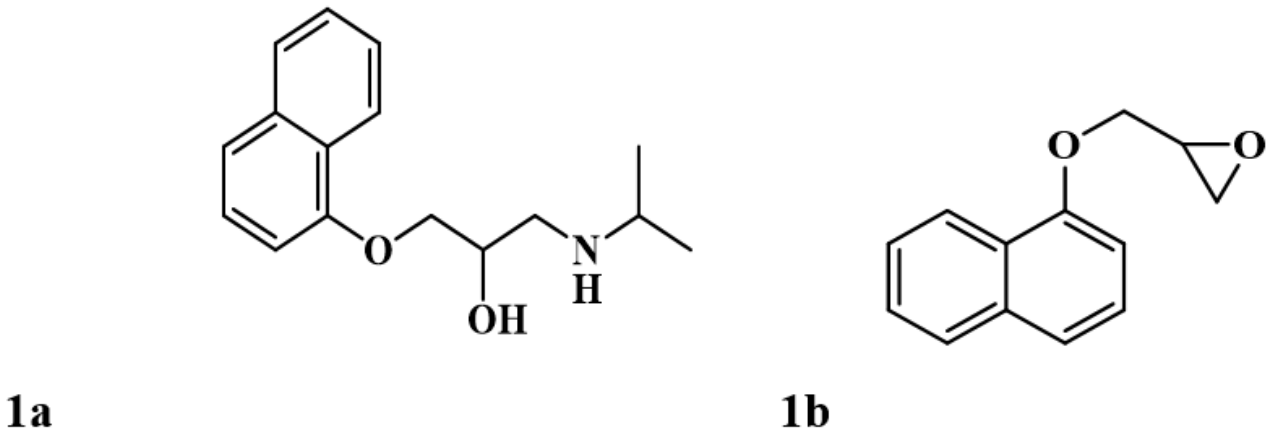


Figure 1a: Lead API use for impurity synthesis (Propranolol). 1b: 2-[(naphthalen-1-yloxy) methyl] oxirane (Intermediate).

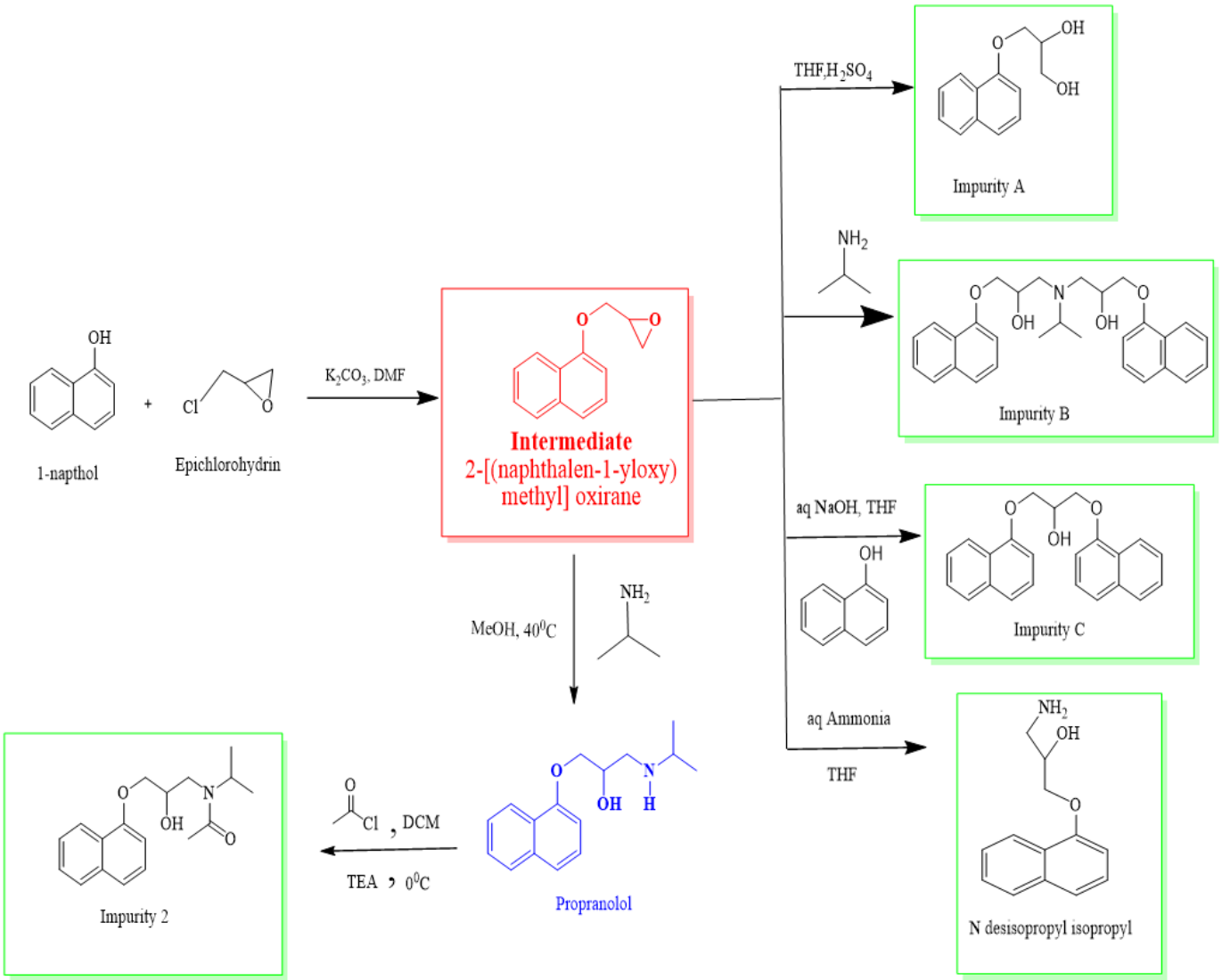


Figure 2: The scheme describes the impurities synthesis of propranolol.

CHARACTERIZATION OF COMPOUNDS

Spectral Data of Propranolol impurity A (3-(naphthalen-1-yloxy) propane-1,2-diol)

^{1}H-NMR-(400MHz, $CDCl_3$), 8.19-8.22(d, 1H, CH), 7.72-7.8(d, 1H, CH), 7.36-7.52(m, 3H, CH), 7.36-7.88(d, 1H, CH), 6.77-6.82(d, 1H, CH), 4.23-4.27(d,2 H, CH_2), 3.8-4.0(m, 2H, CH_2), 4.2-4.23(m, 1H, CH), 2.7(s, 1H, OH), 2.15(s, 1H, OH), MS calculated for $C_{13}H_{14}O_3$: 200.2 g/mol, experimental: Mz=200.20(M^+), 201.20(M+H).

Spectral Data of Propranolol impurity B (3,3'-(isopropyl azanediyl) bis(1-(naphthalen-1-yloxy) propan-2-ol) hydrochloride

^{1}H-NMR (DMSO), 8.22-8.24(d, 1H, CH), 7.83-7.85(d, 1H, CH), 7.29-7.52(m, 3H, CH), 7.25-7.29(d, 1H, CH), 6.81-6.89(d, 1H, CH), 4.01-4.07(d, 2H, CH_2), 2.72-2.74(m, 1H, CH), 2.5-2.74(m, 2H, CH_2), 5.01(s, 1H, OH), 0.89-0.97(m, 3H, CH_3),2.59-2.6(d, 1H, CH), MS calculated for $C_{29}H_{33}NO_4$: 459.6 g/mol, experimental: Mz=459.2(M^+), 460.2(M+H).

Spectral Data of Propranolol impurity C (1,3-bis(naphthalen-1-yloxy) propan-2-ol)

^{1}H-NMR-(400 MHz, $CDCl_3$), 8.25-8.27(d, 1H, CH), 7.80-7.82(d, 1H, CH), 7.47-7.52(m, 3H, CH), 7.25-7.46(d, 1H, CH), 6.88-6.90(d, 1H, CH), 4.4-4.7(d, 2H, CH_2), 4.71-4.73(m, 1H, CH), 2.74-2.81(s, 1H, OH), MS calculated for $C_{23}H_{20}O_3$**:** 344.4 g/mol, experimental: Mz=344.27(M^+), 345.27(M+H).

Spectral Data of Propranolol impurity 2

^{1}H-NMR-($CDCl_3$), 8.19-8.22(d, 1H, CH), 7.80-7.83(d, 1H, CH), 7.29-7.52(m, 3H, CH), 7.36-7.38(d, 1H, CH), 7.85-7.86(d, 1H, CH), 4.21-4.25(t, 2H, CH_2), 3.73-3.79(m, 1H, CH), 4.02-4.1(m, 2H, CH_2), 2.22(m, 3H, CH_2), 3.5-3.56(s, 1H, OH),1.35-1.36(d, 1H, CH),1.20-1.22(m, 3H, CH_3), MS calculated for $C_{18}H_{23}NO_3$**:** 301.4 g/mol, experimental: Mz=301.2(M^+), 302.2(M+H).

Spectral Data of impurity N Desisopropyl Propranolol

^{1}H-NMR (DMSO), 8.21-8.23(d, 1H, CH), 7.85-7.87(d, 1H, CH), 7.37-7.53(m, 4H, CH), 6.94-6.96(d, 1H, CH), 4.05-4.12(m, 2H, CH, CH_2), 2.71-2.88(m, 2H, CH_2), 3.92-3.94(s, 1H, OH), 1.82(s, 2H, NH_2).MS calculated for $C_{17}H_{15}NO_2$**:** 217.11g/mol, experimental: Mz=217.4(M^+), 218.4(M+H).

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

CONCLUSION

The successful synthesis and characterization of five Propranolol Impurities underline their importance as reference standards in pharmaceutical research. Given Propranolol's role in treating cardiovascular diseases, these impurities have high market value and are essential for ensuring the safety and efficacy of the final drug product. Their accurate identification contributes significantly to quality control and regulatory compliance in the drug development process.

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We express our gratitude to Anasyn Lab Pvt. Ltd. for furnishing all the essential synthetic requirements and analytical details of the compound, as well as for their invaluable support and cooperation throughout the execution of this project**.**

ABBREVIATIONS

API: Active Pharmaceutical Ingredient; **EP:** European Pharmacopoeia; **DMF** Dimethylformamide; **TLC:** Thin Layer Chromatography; **THF:** Tetrahydrofuran; **NMR:** Nuclear Magnetic Resonance; **IR:** Infrared.

CONFLICT OF INTEREST

The authors declared there is no conflict of interest.

SUMMARY

Impurities are inherently present during the synthesis of drugs and Active Pharmaceutical Ingredients (API), even at trace levels. These chemically derived impurities are valuable in the pharmaceutical industry, particularly as reference standards. In this study, five Propranolol impurities- Propranolol Impurity-A, Propranolol Impurity-B, Propranolol Impurity-C, Propranolol Impurity-2 and N-Desisopropyl Impurity were synthesized. Propranolol, a widely used cardiovascular drug, holds significant pharmaceutical importance. The purification of these impurities was achieved using column chromatography, while their identities were confirmed through a dual- method approach involving single-spot Thin-Layer Chromatography (TLC) and spectral analysis techniques such as Infrared (IR), Proton Nuclear Magnetic Resonance (^{1}H NMR), and Mass Spectroscopy.

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