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Original Research Article

Synthesis, isolation, identification and characterization of new process-related impurity in isoproterenol hydrochloride by HPLC, LC/ESI-MS and NMR

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ABSTRACT

One unknown impurity (Imp-II) during the analysis of laboratory batches of isoproterenol hydrochloride was detected in the level ranging from 0.04% to 0.12% by high performance liquid chromatography with UV detection. The unknown impurity structure was proposed as 4-[2-(propan-2-ylamino)ethyl]benzene-1,2-diol (Imp-II) using the liquid chromatography–mass spectrophotometry (LC–MS) analysis. Imp-II was isolated by semi-preparative liquid chromatography from the impurity-enriched reaction crude sample. Its proposed structure was confirmed by nuclear magnetic spectroscopy such as ^{1}H, ^{13}C, DEPT (1D NMR), HSQC (2D NMR) and infrared spectroscopy (IR), and retention time and purity with HPLC followed by the chemical synthesis. Due to less removable nature of Imp-II during the purification, the synthetic process was optimized proficiently to control the formation of Imp-II below to the limit < 0.12% in the course of reaction. The new chemical route was developed for the preparation of this impurity in required quantity with purity to use as reference standard. The most probable mechanism for the formation of Imp-II was discussed in details.

1. Introduction

Optically active arylethanolamines are an important class of bioactive compounds widely used as class-II β-blocker, and class-III antiarrhythmic, adrenergic, anthelmintic and antidepressant agents [1]. Isoproterenol hydrochloride (Unites States Adopted Name (USAN)), chemically 3,4-dihydroxy-α-[(isopropylamino)methyl]benzyl alcohol hydrochloride **(1)** (Fig. 1A), is one of the most active sympathomimetic amines developed by Hospira Inc. (Illinois, United States of America). The drug was approved by the Food and Drug Administration (FDA) as a non-selective β-adrenergic agonist and trace-amine associated receptor 1 (TAAR1) agonist under the trade name, Medihaler-Iso and Isuprel, in January 1982. It is used mostly to treat bradycardia, heart block, chronic obstructive pulmonary diseases and rare in asthma, and as an active bronchodilator [2–4].

Several methods have been reported to identify isoproterenol hydrochloride and its metabolites in biological samples such as high performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS) [5,6], gas-liquid chromatography (GLC) [7] and poly(vinylchloride) (PVC) membrane selective electrode [8], selective separation using bismuth silicate ion-exchanger [9] and chemiluminescence determination using luminol-diperiodatoargentate(III) [10]. A sensitive spectrophotometric method recently has been developed for the determination of isopropyl amine, a core moiety of isoproterenol hydrochloride, at the trace level in pharmaceutical drug substances [11].

Through our continuous efforts on the development and optimization of processes for production of active pharmaceutical ingredients (APIs) in bulk scale, a new route was developed for the preparation of isoproterenol hydrochloride. The final step in the preparation of isoproterenol hydrochloride involves the de-benzylation, subsequently, reduction of keto group to hydroxyl function when 1-(3,4-bis(benzyloxy)phenyl)-2-(isopropylamino)ethanone hydrochloride **(2)** was treated with Pd/C catalyst to afford drug molecule **1** as shown in Fig. 1A. It was found that two processes related impurities named Imp-I and Imp-II consistently in HPLC analyses of isoproterenol hydrochloride were obtained from laboratory batches. The co-spiking analysis and molecular weights found in LC–MS revealed that Imp-I was harmonized with reported impurity, isoproterenone and Imp-II did not match with

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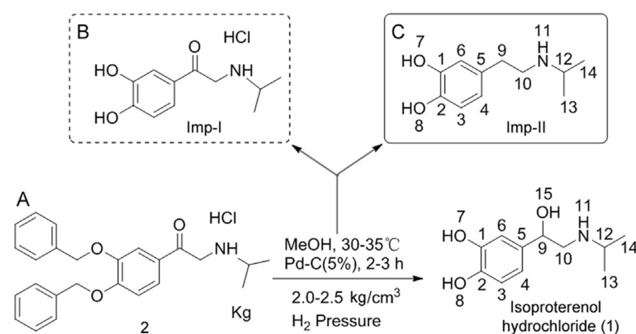

Fig. 1. (A) The final step of development process, (B) chemical structure of reported impurity (Imp-I) and (C) chemical structure of new impurity (Imp-II).

any impurity, and was considered to be unknown.

The impurities in drug molecule can show significant impact on the quality and safety of the drug product. The regulatory authorities have paid considerable attention to controlling impurities in drug molecule to promote it for market approval [12]. International Conference on Harmonization (ICH) recommended guidelines to qualify the drug substance [13]. The impurities are ≥0.1%, concerning the stringent purity requirement, or else it should be identified and characterized [13,14]. Therefore, process development of the drug molecules without impurity profiling is scant and will be a challenging task for organic chemists. Many reports displayed the approach for the identification and characterization of unknown impurities formed in the drug development process [15,16]. In addition, some of the impurities are not available readily and would be essential in required quantity for method development and validation. Therefore, the synthesis of impurities is not an easy task for the development team since the synthesis approach is not known or described in the literature. Our group has documented well the impurity profiling of vildagliptin, ticagrelor, acrivastine and clobazam including the synthesis of impurities [15–18]. However, extensive literature search disclosed that no liquid chromatography methods have been developed so far for the identification of impurities in isoproterenol hydrochloride.

Considering the overview facts and our continuing efforts on impurity profiling [15–18], considerable attention has been focused on identifying and characterizing the unknown impurity in isoproterenol hydrochloride by analytical applications. In addition, the new impurity, Imp-II was prepared by chemical synthesis with purity by avoiding tedious work-up procedure and laborious column chromatography techniques to use as a reference standard in analytical method development. To the best of knowledge, detection, separation, characterization, synthesis and plausible mechanism to the formation of impurities in isoproterenol hydrochloride are first reported in detail.

2. Materials and methods

2.1. Materials and reagents

The samples of isoproterenol hydrochloride obtained from different final step batches, reference standard of isoproterenol hydrochloride (purity 99.9%) and the starting material, 1-(3,4-bis(benzyloxy)phenyl)-2-(isopropylamino)ethanone hydrochloride **(2)** used in the synthesis, were provided by Chemical Research Division, Micro Labs Ltd. (Bangalore, India). The catalyst, Pd-C (50% wet), was acquired from Monarch Catalyst Private Limited (Mumbai, India). HPLC grade acetonitrile and methanol were purchased from Spectrochem Pvt Ltd. (Bangalore, India), and formic acid and trifluoroacetic acid were purchased from Sigma-Aldrich (Bangalore, India). The commercial grade ethyl acetate (EtOAc) and isopropyl alcohol (IPA) were procured from Taiwan Fieldrich Corporation (Taipei, China). Purified water by Millipore Milli-Q Plus Purification System (Bradford, PA, USA) was used during the course of experimental studies. CD_3OD was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Potassium bromide used in Fourier transform infrared spectroscopy (FT-IR) was purchased from Merck KGaA (Darmstadt, Germany). TLC plates (60 F_{254}) procured from Merck (Delhi, India) was used to check the progress of reaction.

2.2. HPLC

The chromatographic experiments were carried out on Nexera-X2 model ultra-high performance liquid chromatography technique (UHPLC) (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array detector (SPD 20A) and SPD M30 dual wavelength absorbance detector. The Empower Software was utilized for monitoring the process, data acquisition and system control. The test sample solutions were prepared in diluent, a mixture of methanol (MeOH)-water (20:80, v/v). The related substances of isoproterenol hydrochloride were carried out on a reverse phase Agilent Zorbax Rx C_8 (250 mm × 4.6 mm, 5 µm) column (Agilent Technologies, CA, USA) by maintaining column temperature at 40 °C. Mobile phase A was trifluoroacetic acid (0.1%) solution which was prepared by dissolving 1.0 mL of trifluoroacetic acid in 1000 mL of water, adjusted the pH to 3.0 ± 0.05 with triethylamine, and acetonitrile was used as mobile phase B. The injection volume and wavelength were fixed at 5 µL and 280 nm, respectively, and the data were acquired by using gradient elution system at a flow rate of 1.0 mL/min. The separation was accomplished by employing a linear gradient programme as T_{min}/A:B: T_0/95:5, T_5/90:10, T_{10}/90:10, T_{25}/15:85, T_{35}/15:85, and T_{36}/95:5 with an equilibration time of 1.0 min.

2.3. LC–MS

The MS analyses were performed on a Velos Pro ion trap mass spectrophotometer from Thermo Scientific (San Jose, CA, USA) using Thermo X-Caliber, version 2.2, software. The instrument was operated using electrospray ionization source (positive ion mode), with source voltage at 4.0 kV, spray current at 100.0 µA, desolvation temperature at 200 °C and capillary temperature at 300 °C. Nitrogen gas was used for desolvation and as tube lens gas.

The chromatographic separation was carried out on Nexera-X_2 (Shimadzu, Kyoto, Japan) HPLC equipped with photodiode array detector using Thermo Accucore MS C_{18} (150 mm×4.6 mm, 2.6 µm) column (Thermo Scientific, San Jose, USA) by maintaining column temperature at 25 °C. Mobile phase A (0.1% aqueous trifluoroacetic acid) and mobile phase B (methanol) were used for elution of the components. The separation was attained by following the gradient programme as (T_{min}/A:B) T_0/90:10, $T_{0.5}$/90:10, $T_{1.5}$/80:20, $T_{6.0}$/70:30, $T_{10.0}$/40:60, $T_{12.0}$/10:90, $T_{20.0}$/10:90, $T_{21.0}$/90:10 and $T_{25.0}$/90:10 with a flow rate of 0.6 mL/min. The eluent was passed through MS analyzer for acquisition of data.

2.4. Semi-preparative liquid chromatography

Shimadzu Prominence LC20AP (Shimadzu Corporation, Tokyo, Japan) equipped with LC-20AP binary gradient module, SIL-10AP sample manager, and SPD-M20A PDA detector was used to isolate the impurity. The data were processed through Lab Solution Software. The column, InertSustainSwift C_{18} (250 mm×20 mm, 5 µm) column (G L Sciences, Eindhoven, Netherlands), was used to attain chromatographic separation. The sample concentration of 200 mg/mL was prepared in diluent. Aqueous acetic acid (0.1%) and acetonitrile were used as mobile phases A and B, respectively. The desired impurity was obtained by eluting mobile phase A-mobile phase B (95:5, v/v). The eluent was monitored at 280 nm. The collected fractions were lyophilized using lyophilizer.

2.5. Enrichment and isolation of Imp-II

1-(3,4-Bis(benzyloxy)phenyl)-2-(isopropylamino)ethanone hydrochloride **(2)** (10.0 g, 0.0235 mol) and the catalyst, Pd-C (50% wet) (2.0 g, 20% m/m) in MeOH (140 mL), were charged into a 500 mL autoclave (Fig. S1). The reaction mixture was degassed by evacuating and refilling via N_2 three times, and then heated to 50–55 °C. The reaction mixture was agitated about 6.0 h by maintaining H_2 pressure at 5.0–6.0 kg/cm^3. HPLC analysis indicated 13.6% formation of Imp-II. The reaction mass was cooled to room temperature, unloaded from autoclave, filtered under nitrogen atmosphere through celite to remove the catalyst (Pd-G). The bed was washed with MeOH (20 mL). The combined filtrate was concentrated under vacuum at 45 °C to obtain crude samples. Semi-preparative HPLC was employed to isolate the desired impurity from the crude samples. Imp-II was eluted between 3.42 and 3.51 min. The corresponding fractions were collected, combined and lyophilized using lyophilizer. The Imp-II about 796 mg was obtained as off-white solid with purity of 98.39% as checked by HPLC.

2.6. Nuclear magnetic resonance spectroscopy (NMR)

^{1}D NMR (^{1}H, ^{13}C and Distortionless Enhancement by Polarization Transfer Spectroscopy (DEPT)) and ^{2}D NMR (Heteronuclear Single Quantum Coherence Spectroscopy (HSQC)) experiments were performed on an Ascend™ Bruker 400 MHz NMR spectrometer (Bruker, Fallanden, Switzerland) using deuterated methanol (CD_3OD) as a solvent and tetramethylsilane (TMS) as an internal standard. DEPT spectral editing was used to identify methyl and methine groups as positive peaks and methylene as negative peaks, and HSQC experiment was carried out for assignment of the related chemical shift values. The ^{1}H chemical shift values were reported on δ scale in parts per million (ppm), relative to TMS (δ=0.00 ppm) and the ^{13}C chemical shift values were reported relative to CD_3OD (δ=49.3 ppm). Isoproterenol hydrochloride and Imp-II were numbered as depicted in Fig. 1 to assign the proper spectral characterization.

2.7. Fourier transform infrared spectroscopy (FT-IR)

The infrared spectroscopy data of isoproterenol hydrochloride and Imp-II were recorded on a Shimadzu IR Affinity-I FT-IR spectrophotometer (Kyoto, Japan) over the range of 4000–400 cm^{-1} by pressed pellet method using KBr. The spectra were acquired by accumulation of 42 scans with 4 cm^{-1} resolution. The absorption values were represented in cm^{-1}.

2.8. Melting point measurement

The melting range of compounds was recorded on Digital BUCHI apparatus (BUCHI Corporation, Flawil, Switzerland) and M-565 model.

2.9. Synthesis of Imp-II

The mixture of Zn dust (15.37 g, 0.235 mol), $HgCl_2$ (0.96 g, 3.525 mmol) and 2 M HCl solution (20 mL) was taken into a round bottom flask and stirred vigorously about 1.0 h at ambient temperature. The suspension solid was separated by filtration and the lumps were broken, resulting in Zn-Hg amalagum. Immediately, the mixture of 1-(3,4-bis(benzyloxy)phenyl)-2-(isopropylamino)ethanone hydrochloride **(2)** (10.0 g, 0.0235 mol), formic acid (20 mL) and Zn-Hg amalagum solid was taken into a vessel containing methanol (20 mL). The reaction mixture was heated to 65–70 °C, and stirred about 8.0 h, and TLC analysis confirmed the reaction completion. The reaction mass was cooled to room temperature and filtered through celite bed. pH of the filtrate was adjusted to 8–9 with 10% Na_2CO_3 solution (50 mL) and the organics were extracted two times with ethyl acetate (100 mL × 2). The combined ethyl acetate solution was washed with brine (saturated NaCl) solution (50 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure at 50 °C to afford crude product, *N*-(3,4-bis(benzyloxy)phenethyl)propan-2-amine as a thick mass. Isopropyl alcohol hydrochloride (15%) solvent (50 mL) was added to this thick mass and then stirred for 3.0 h at room temperature. The solid product was separated by filtration, and the solid bed was washed with IPA (10 mL). The final solid product was dried to obtain 8.82 g (90.94%) of the product, *N*-(3,4-bis(benzyloxy) phenethyl)propan-2-amine hydrochloride **(3)**.

The compound, *N*-(3,4-bis(benzyloxy)phenethyl)propan-2-amine hydrochloride **(3)** (7.0 g, 0.01866 mol) and the catalyst, Pd-C (50% wet) (350 mg, 5% m/m), in MeOH (98 mL) was charged into a 500 mL autoclave. The reaction mixture was degassed by evacuating and refilling via N_2 three times and then heated to 30–35 °C. The reaction mixture was agitated for 2.0 h by maintaining H_2 pressure at 2.0–2.5 kg/cm^3. HPLC analysis confirmed the reaction completion (98% of product). The reaction mass was unloaded from autoclave and filtered off through celite bed under nitrogen atmosphere to remove the catalyst, Pd-C. The collected filtrate was concentrated under reduced pressure at 45 °C and the crude product was purified by recrystallization with ethanol to obtain desired pure product, 4-(2-(isopropylamino)ethyl)benzene-1,2-diol hydrochloride **(4)** (3.64 g, 98.56%), which is off-white solid, m.p.: 188–190 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 1.34 (d, *J* =6.8 Hz, 6H, H-13 & H-14), 2.84 (t, *J* =8.4 Hz, 2H, H-9), 3.15 (t, *J* =8.4 Hz, 2H, H-10), 3.36–3.43 (m, 1H, H-12), 6.60 (dd, *J* =2.0, 6.0 Hz, 1H, H-4), 6.73 (m, 1H, H-3), 6.76 (s, 1H, H-6) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ 19.22 (C-13 & C-14), 33.03 (C-9), 47.60 (C-10), 51.95 (C-12), 116.74 (C-6), 116.80 (C-3), 120.99 (C-4), 129.03 (C-5), 145.69 (C-2), 146.81 (C-1) ppm; ESI-MS (positive mode) *m/z* (%): 196.08 (M + H$^+$) (100%), 192.08 (M + H$^+$− 4) (6%), 137.00 (M + H$^+$− 59) (18%).

3. Results and discussion

3.1. Detection of unknown impurity

Different isoproterenol hydrochloride samples obtained from lab batches were analyzed by typical HPLC method with UV detection. Two impurities at relative retention time (RRT) of 1.11 (retention time (RT) of 5.61 min) and 1.55 (RT of 5.80 min) in the percentage peak area of 0.01%–0.06% and 0.04%–0.12%, were observed and marked as Imp-I and Imp-II, respectively (Fig. 2). One process-related impurity of isoproterenol hydrochloride was reported in the literature as in the name of isoproterenone [19] (Fig. 1B). For sake of convenience, the synthesized impurity was spiked with isoproterenol hydrochloride and it was observed that Imp-I RT (5.61 min) exactly concurred with reported impurity RT (5.59 min). Therefore, the structure of Imp-I was confirmed as isoproterenone or (1-(3,4-dihydroxyphenyl)-2-(isopropylamino)ethanone hydrochloride) (Fig. 1B). Based on the synthetic knowledge, we speculate the cause for the appearance of Imp-I is due to no entire transformation of debenzylated intermediate to a drug molecule **1**. However, Imp-II did not match with any reported impurity or intermediate. The developed HPLC method was not compatible to LC–MS; therefore, the sample was injected to newly established LC–MS system as described in Section 2.3 to examine Imp-II molecular weight. The LC–MS spectrum manifested the protonated molecular ion of isoproterenol hydrochloride (*m/z* 212.14) and Imp-II (196.10) (Fig. 3). The molecular weight of Imp-II did not coincide to any impurity or intermediate used in isoproterenol hydrochloride drug and Imp-II was inferred to be unknown, thus its structure needs to be identified.

3.2. Proposed structural elucidation of Imp-II by LC–MS/ESI

Prior to analysis of Imp-II, the mass spectral fragmentation of the

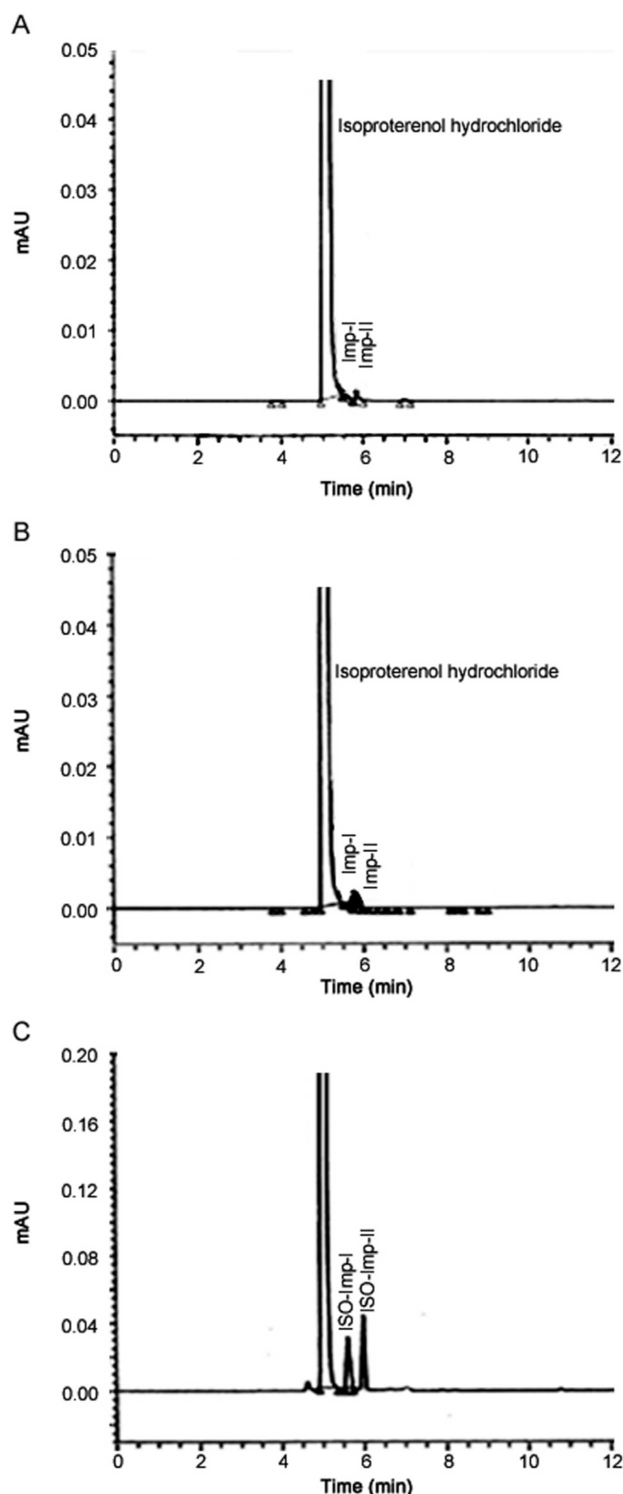

Fig. 2. HPLC chromatogram of (A) isoproterenol hydrochloride after purification, (B) crude mass of isoproterenol hydrochloride before purification, and (C) impurities mixture.

parent drug, isoproterenol hydrochloride was investigated. The ESI mass spectrum of isoproterenol hydrochloride demonstrated molecular ion at *m/z* 212.14 and yielded three major daughter ions at *m/z* 208, 194 and 192 (Fig. S2). The difference of masses from molecular ion to fragmented ion *m/z* 208 was 4.0 and this could be attributed to loss of two neutral hydrogen molecules: one might be from hydroxyl group (–CH-OH) resulting in keto group and the other from two hydroxyl groups on benzene ring resulting in benzoquinone. The yielded fragment ion *m/z* 194, due to loss of water molecule, is conceivable by the loss of alcohol group (–OH) from benzylic position and hydrogen from *para* positioned phenolic group, and finally led to quinonoid form by shifting of the other hydrogen from adjacent phenolic. The product ion *m/z* 192 could be formed by the loss of hydrogen molecule (−2.0 amu) from –NH of isopropyl amine resulting in iminium entity. In ESI spectrum of Imp-II, the protonated molecular ion at *m/z* 196 yielded two daughter ions at *m/z* 194 and 192 (Fig. S3). No fragment was formed by the loss of 18.0 amu like compound **1**; it was postulated that Imp-II did not have aliphatic hydroxyl groups. The fragment at *m/z* 194 might be loss of hydrogen molecule from dihydrox benzene which led to quinonoid form and further loss of hydrogen molecule (−2.0 amu) form –NH like isoproterenol hydrochloride to produce daughter ion *m/z* 192 as iminium scaffold. The hydrogenolysis of benzylic alcohols over palladium catalyst has been reported in the specifics [20–22]. In view of aforesaid observations, the proposed structure of Imp-II was 4-[2-(propan-2-ylamino)ethyl]benzene-1,2-diol. The plausible fragmentation behavior of isoproterenol hydrochloride and Imp-II can be explained by the mechanism given in Fig. 4.

3.3. Isolation and collection of Imp-II

A small quantity of Imp-II formed in the level range of 0.04%–0.12% under optimization condition is very difficult to isolate in the required quantity. Hence, we adopted stress conditions to enrich Imp-II as discussed in Section 2.5 and HPLC analysis revealed that Imp–II was enriched to 13.6%. Then, the semi-preparative HPLC was employed to isolate crude mass, the impurity was eluted at RT 3.48 min, and the fractions were collected manually between 3.42 and 3.51 min. The corresponding fractions were lyophilized using lyophilizer to obtain Imp-II as off-white solid. The isolated Imp-II was co-injected into HPLC system to reanalyse and to check RT and purity. The data revealed that Imp-II was well resolved with reference sample with purity of 98.39% and the RT (5.80 min) of isolated Imp-II was coincided exactly with crude sample. Hence, this isolated solid was used directly to confirm the proposed structure through spectral characterization without any further purification.

3.4. Structural confirmation of Imp-II

In order to confirm the structure of Imp-II, the nuclear magnetic resonance spectroscopic profile such as ^{1}H NMR, ^{13}C NMR, DEPT, HSQC and IR were predicted. To distinguish optimum characterization of Imp-II, the same spectroscopic profile was recorded to the drug molecule **1**. ^{1}H NMR, ^{13}C NMR, DEPT data of isoproterenol hydrochloride and Imp-II are shown in Table 1 and their HSQC correlated data in Table 2.

In ^{1}H NMR spectrum of Imp-II (Fig. S4), we did not find any significant deviation in the chemical shift values of aromatic and isopropyl entity protons as compared to isoproterenol hydrochloride (Fig. S5) except at H-9 and H-10 protons. The proton, H-9, was shielded to 2.84 ppm in Imp-II while compared with isoproterenol hydrochloride as 4.84 ppm; however, this chemical shift value was closer to benzylic protons and two protons were found instead of single proton. These findings suggested that the functionality –CH-OH in isoproterenol hydrochloride was converted into $–CH_2$ to form Imp-II.

Noteworthy variation was observed at C-9 and C-10 in Imp-II (Fig. S6) as compared with isoproterenol hydrochloride (Fig. S7) in ^{13}C spectra. Extremely, C-9 carbon was shielded to 33.00 ppm in Imp-II while compared with isoproterenol hydrochloride as 70.20 ppm and this promising deviation might be the absence of electronegative oxygen atom attachment. The DEPT spectrum (Fig. S8) has given clear evidence to $–CH_2$ formation in Imp-II. In isoproterenol hydrochloride, one negative peak at 52.57 ppm indicates $–CH_2$ (C-10). In contrast, two negative peaks at 46.15 and 31.61 ppm appeared in Imp-II and remained carbons approximately like isoproterenol hydrochloride

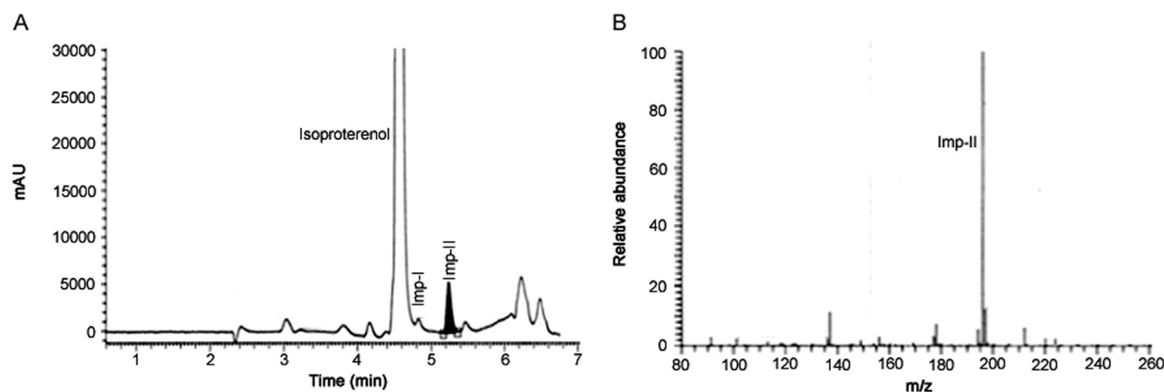

Fig. 3. LC–MS chromatogram of (A) crude isoproterenol hydrochloride and (B) mass spectrum of Imp-II.

except the peak at 70.20 ppm. The appearance of new negative peak at 31.61 ppm and no positive peak at 70.20 ppm corresponding to –CHOH group in the DEPT spectrum of Imp-II when compared with isoproterenol hydrochloride indicated that Imp-II was formed by the reduction of –CHOH group present in isoproterenol hydrochloride into $-CH_2$ functionality. The C-10, due to the loss of –OH function at C-9, was shifted to shielding 46.15 ppm from 52.57 ppm. In addition, we also recorded $^{1}H-^{13}C$ correlation spectroscopy such as HSQC. It provides the correlation of carbons and directly attached proton. In HSQC spectrum (Fig. S9), the carbons at 19.24 ppm (C-13 and 14) bonded with six protons are resonated at 1.34 ppm as doublet, carbon at 33.00 ppm (C-9) connected with two protons are resonated at 2.84 ppm as triplet, carbon at 47.61 ppm attached with two protons are resonated at 3.15 ppm as triplet and another aliphatic carbon at 51.95 ppm bonded with one proton is resonated at 3.36–3.43 ppm as multiplet. The aromatic carbons appeared at 116.76 ppm (C-6), 116.83 ppm (C-3) and 121.03 ppm (C-4) associated with one proton appeared at 6.76 ppm as singlet, 6.73–6.74 ppm as multiplet and 6.60–6.63 ppm as multiplet, respectively. Based on the spectroscopic analysis, the proposed structure of Imp-II was confirmed as 4-(2-(isopropylamino)ethyl)benzene-1,2-diol (Fig. 1C).

In FT-IR spectrum of Imp-II (Fig. S10), the absorption bands at 3375 cm^{-1}, 3317 cm^{-1} and 3209 cm^{-1} indicated the functionalities, two OH and –NH, respectively. We did not observe the significant variations in Imp-II while comparing with isoproterenol hydrochloride (Fig. S11).

3.5. Formation of Imp-II

The prospects to form Imp-II were proposed based on the structure of impurity and synthetic knowledge on the specific step. In addition, a number of mechanistic studies concerning the catalytic hydrogenolysis of benzyl alcohol derivatives over palladium have been reported [20–22]. Kieboom et al. [22] reported that the displacement of primary alcoholic group in benzylic system occurs in S_N2 type and tertiary alcoholic group in S_N1 type. They also proved by Hammett relation that the presence of substituents in the 3- and 4-positions of the aromatic ring, during the hydrogenolysis of alcohol group, established electron deficient transition state with partially charged, which will be stabilized by aromatic ring. It indicated that the hydrogenolysis or displacement of alcohol was ensued through S_N2 type mechanism by the hydride ion attacking provided by palladium. In the same fashion, the hydroxyl group substituted at *p*-position of the aromatic ring in isoproterenol hydrochloride might provide partially electron deficient center resulting in quinonoid type transition state, simultaneously, the hydride ion attacking from palladium at deficient carbon center led to formation of Imp-II. The most probable mechanistic pathway to form Imp-II is depicted in Fig. 5.

3.6. Control of Imp-II

As discussed above, Imp-II is formed by involving the drug molecule **1** into a further reduction with Pd-C. Imp-II has similar structure with drug molecule expect aliphatic hydroxyl group. Due to its comparable solubility in ethanol and yield loss of target drug molecule, the purification process with ethanol is unsuccessful to accomplish to the desired limit (0.10%) while forming Imp-II in high quantity in the reaction. Therefore, Imp-II removal in purification is challenging; we controlled the Imp-II in the reaction process with a limit of not more than 0.12%. This level of Imp-II in the crude mass could be suppressed and further purification was needed to achieve acceptable level ~ < 0.06%. The Imp-II formed was not more than the specified limit under optimized conditions as shown in the final step (Fig. 1A). The firmly followed precautions, during the progress of reaction, to avoid or control the formation of Imp-II include (i) the H_2 pressure has to below 2.5 kg/cm^3, (ii) temperature has to maintained below 35 °C, (iii) during HPLC sampling, the agitation should be stopped and the H_2 in container be removed and flushed with N_2.

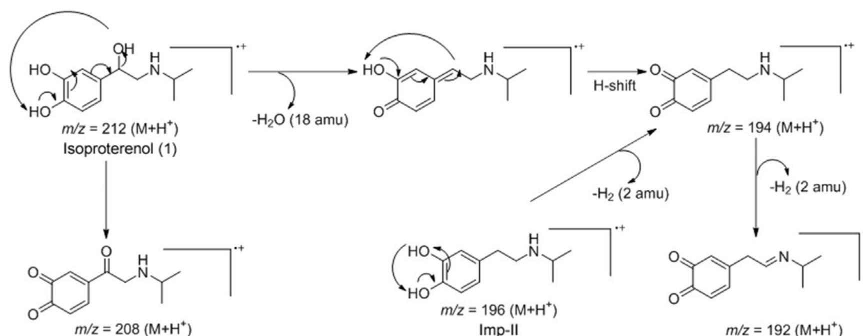

Fig. 4. The plausible mass fragmentation pathway of isoproterenol hydrochloride and Imp-II.

Table 1
Comparative ^{1}H NMR, ^{13}C NMR and DEPT assignments of isoproterenol hydrochloride and Imp-II.

Position	Isoproterenol				Impurity II				Imp II deviation	
	Structural part	^{1}H NMR ppm/multiplicity/J	^{13}C NMR	DEPT	Structural part	^{1}H NMR ppm/multiplicity/J	^{13}C NMR	DEPT	$\Delta\delta_H$	$\Delta\delta_C$
1	HO-C in Ar	–	146.52	–	HO-C in Ar	–	146.75	–	–	0.23[*]
2	HO-C in Ar	–	146.59	–	HO-C in Ar	–	145.63	–	–	0.96[*]
3	–CH in Ar	6.79/d/1H/8.0 Hz	116.44	116.43[b]	–CH in Ar	6.73–6.74/m/1H	116.83	115.33[b]	0.06[*]	0.39[*]
4	–CH in Ar	6.76–6.78/dd/1H/ 1.2 & 6.8 Hz	118.56	118.56[b]	–CH in Ar	6.60–6.63/dd/1H /2.0 & 6.0 Hz	121.03	119.51[b]	0.16[*]	2.47[*]
5	–CH-C in Ar	–	133.86	–	–CH-C in Ar	–	129.12	–	–	4.74[**]
6	–CH in Ar	6.91/s/1H	114.21	114.21[b]	–CH in Ar	6.76/s/1H	116.76	115.29[b]	0.15[*]	2.55[*]
7	Ar-OH	–	–	–	Ar-OH	–	–	–	–	–
8	Ar-OH	–	–	–	Ar-OH	–	–	–	–	–
9	Ar-CH(OH)	4.84/t/1H/6.8 Hz	70.20	70.20[b]	Ar-CH_2	2.84/t/2H/8.4 Hz	33.00	31.61[a]	2.00[**]	37.2[**]
10	–CH_2-NH–	3.04–3.15/m/2H	52.57	52.57[a]	–CH_2-NH–	3.15/t/2H/8.4 Hz	47.61	46.15[a]	0.11[*]	4.96[**]
11	–NH–	–	–	–	–NH–	–	–	–	–	–
12	–CH(CH_3)$_2$	3.42–3.47/m/1H	51.94	51.93[b]	–CH(CH_3)$_2$	3.36–3.43/m/1H	51.95	50.51[b]	0.06[*]	0.01[*]
13 & 14	–CH(CH_3)$_2$	1.35–1.38/2d/ 6H/6.4 Hz	18.86 & 19.43	18.85 & 19.43[b]	–CH(CH_3)$_2$	1.34/d/ 6H/6.8 Hz	19.24	17.77[b]	0.01[*]	0.38[*]
15	–OH	–	–	–	–	–	–	–	–	–

Highlighted: a position part in the molecule;
[a] negative peaks in DEPT.
[b] positive peaks in DEPT.
[*] no significant deviation.
[**] significant deviation to shielding.

Table 2
H-C correlation of isoproterenol and Imp-II by HSQC.

Isoproterenol			Impurity II		
δ_H/δ_C	Correlation assignment	Structure	δ_H/δ_C	Correlation assignment	Structure
1.35/18.86 1.38/19.43	H-13 and 14/ C-13 and 14, two CH_3 in isopropyl chain		1.34/ 19.24	H-13 and 14/ C-13 and 14, two CH_3 in isopropyl chain	
3.04/52.57	H-10/C-10, CH_2 group in between CH(OH) and NH		2.84/ 33.00	H-10/C-10, CH_2 group in between CH_2 and phenyl ring	
3.42/51.94	H-12/C-12, CH group in isopropyl chain		3.15/ 47.61	H-12/C-12, CH_2 group in between CH_2 and NH	
4.84/70.20	H-9/C-9, aliphatic CH group linked to –OH		3.36/ 51.95	H-9/C-9, CH group in isopropyl chain	
6.76/118.56	H-4/C-4, in phenyl ring		6.60/ 121.03	H-4/C-4, in phenyl ring	
6.79/116.44	H-3/C-3, in phenyl ring		6.73/ 116.83	H-3/C-3, in phenyl ring	
6.91/114.21	H-6/C-6, in phenyl ring		6.76/ 116.76	H-6/C-6, in phenyl ring	

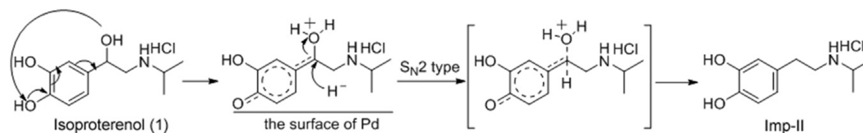

Fig. 5. Plausible mechanism for the formation of Imp-II.

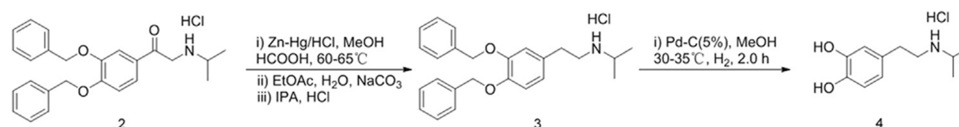

Fig. 6. Synthesis of compound **4** or Imp-II.

3.7. Synthesis of Imp-II

The development of new synthetic processes to the preparation of impurities is also considered an interesting strategy in synthetic chemistry. Hence, a simple new synthetic route was established for the synthesis of Imp-II as shown in Fig. 6.

Initially, Zn-Hg amalgam was prepared by the treatment of Zn dust with $HgCl_2$ in the presence of HCl solution. Then, the freshly prepared Zn-Hg amalgam was treated with 1-(3,4-bis(benzyloxy)phenyl)-2-(isopropylamino)ethanone hydrochloride **(2)** in methanol using formic

acid as catalyst to obtain crude compound, *N*-(3,4-bis(benzyloxy) phenethyl)propan-2-amine **(3)**. The hydrochloride salt of compound **3** was prepared by treating with HCl in isopropyl alcohol. Finally, the desired 4-(2-(isopropylamino)ethyl)benzene-1,2-diol **(4)** was obtained by de-benzylation of compound **3** in the presence of Pd-C (5%) catalyst and H_2 pressure in methanol. The catalyst was removed by the filtration and the filtrate was concentrated to obtain crude compound **4**; subsequently, it was recrystallized with ethanol to obtain the pure compound as off-white solid. The compound was reanalysed by HPLC to check its purity and RT. The RT of compound **4** exactly matched with Imp-II with 97.8% purity. The synthesized compound **4** was characterized by spectroscopic analyses such as ^1H NMR, ^{13}C NMR and MS analysis (Figs. S12–S14), and the data concurred with the data of Imp-II.

4. Conclusion

One unknown process-related impurity (Imp-II) was identified in isoproterenol hydrochloride using HPLC with UV detection. The impurity was isolated by semi-preparative liquid chromatography from impurity enriched crude sample. The spectroscopic analyses, such as IR, ^1H NMR, ^{13}C NMR, DEPT, and HSQC, and mass analysis were performed to elucidate the structure of the compound accurately and the compound was confirmed as 4-(2-(isopropylamino)ethyl)benzene-1,2-diol. The most reasonable mechanism for formation of Imp-II and its controlling during the synthesis of isoproterenol hydrochloride were discussed. In addition, a synthetic route for the preparation of Imp-II in high yield with purity was established by adopting simple workup procedure, and crystallization, thus avoiding laborious time taken by column chromatography. The findings in the present study show promising future to analytical and synthetic chemistry that (i) the identified unknown impurity could keep in the specification of isoproterenol hydrochloride as a known impurity, (ii) the approach would be useful to find the unknown impurities in the development of drug molecules and (iii) we could carry out further pharmacological or toxicological study to distinguish bio-efficacy of this impurity (Imp-II).

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpha.2017.07.003.

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