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Practical synthetic approach to related substances of Rivaroxaban; an anticoagulant drug substance

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Abstract

During the process development of an anticoagulant drug, Rivaroxaban (1), three related substances were detected by a gradient high performance liquid chromatography (HPLC) method. Liquid chromatography mass spectrometry (LC-MS) was performed to identify the molecular mass of these impurities. A detailed study was undertaken to characterize these impurities. Based on the spectral data (^{1}H NMR, ^{13}C NMR and MS), these impurities were characterized as 2-[(2S)-2,3-dihydroxypropyl]-1H-indene-1,3(2H)-dione (impurity-1), [2-({4-[(5S)-5-({[(5-chloro-2-thienyl)carbonyl]amino}methyl)-2-oxo-1,3-oxazolidin -3-yl]phenyl}amino) ethoxy]acetic acid (impurity-2) and 5-chloro-N-[(2R)-2-hydroxy-3-{[4-(3-oxomorpholin-4-yl) phenyl]amino}propyl] thiophene-2-carboxamide (impurity-3). A practical and efficient approach for the synthesis of these impurities with good yields and purities by HPLC is described in this report. The structures of the synthesized impurities (imputiy-1, impurity-2 and impurity-3) were further confirmed by co-injecting these impurities with the standard Rivaroxaban sample containing all the three impurities. The retention times of synthesized impurities matches (co-eluted) with the retention times of the impurities present in the standard sample.

Keywords: Rivaroxaban, Related substances, Anti-coagulant drug, Synthesis**.**

Introduction

Rivaroxaban (1) is highly potent and orally active direct factor Xa (fXa) inhibitor drug developed by Bayer used for treatment and prevention of various thromboembolic diseases, in particular pulmonary embolism, deep venous thrombosis, myocardial infraction, angina pectoris, reocclusion and restenosis after angioplasty or aortocoronary bypass[1-2]. This drug was approved under the trade name of Xarelto® by various regulatory authorities like United States Food and Drug Administration, Health Canada and the European commission[3-4]. In literature[5-6] many HPLC and LC-MS methods has been reported for the determination of potential impurities in rivaroxaban (1) but study towards the identification and synthesis of impurities has not been reported. This impurity profiling study will be of immense importance for process development chemist as well as analytical development chemist to understand the potential impurities in 1 (Figure-1). Routine analysis of drug substance or drug product at Quality Control (QC) department requires sufficient quantities of related substances for quantitative estimation to ensure the control of these impurities before their release as per ICH guidelines[7]. Thus; it is essential and important to establish the facile and robust synthesis for the related substances and their characterization during drug development activity.

Recently we have reported sustainable and efficient process for the production of Rivaroxaban (1) but synthesis and characterization of impurities was not covered[8]. Sample of Rivaroxaban (1) generated during process development was analyzed using the established HPLC method wherein three impurities with area percentage ranging from 0.02 to 0.30% were detected at relative retention times (RRT's) 0.25, 0.37 and 0.81 with respect to retention time of 1. To commercialize an active pharmaceutical ingredient (API), it is mandatory for the manufacturer to identify all the unknown impurities that are present in API at the level of even blow 0.05%.[7] With this background, identification, synthesis and characterization of impurities present in rivaroxaban (1) has been undertaken and the outcome of the study is reported herein.

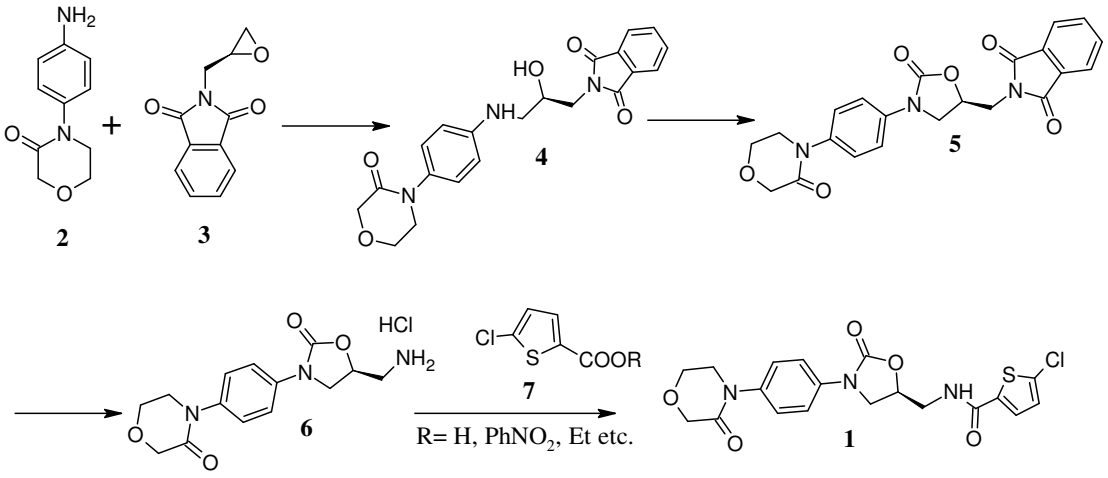
Experimental

All reagents, solvents, and processing aids are commercial products. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-*d6* and $CDCl_3$ using Bruker Avance 300 MHz FT NMR spectrometer; the chemical shifts are reported in δ ppm relative to TMS. LC-MS and mass spectra were performed on Shimadzu Nexara 2020. Related substance purity was monitored by high performance liquid chromatography (HPLC) on Agilent Technologies 1260 series.

HPLC Method for calculating the chemical purity: Column: Zorbax SB-CN, (250 x 4.6 mm ID), 5µ; Mobile phase A: phosphate buffer (0.01M potassium dihydrogen orthophosphate, 0.005 M 1-heptane sulphonic acid sodium salt, triethylamine,

and adjust the pH 6.7 using orthophosphoric acid). Mobile phase B: acetonitrile/water in the ratio 80:20 (v/v); flow rate: 1.5 mL/min; column temperature: 35 °C; wavelength: 240 nm.

Synthesis of 2-[(2S)-2,3-dihydroxypropyl]-1H-indene-1,3(2H)-dione (Impurity-1): To a solution of 2-[(2S)-oxiran-2-ylmethyl]-1H-isoindole-1,3(2H)-dione (3, 10 g, 0.049 mol) in acetonitrile (100 mL), 10% aqueous sodium hydroxide solution (20 mL) was added at 25-30 °C. Reaction mixture was heated to 55-60 °C for 2 h. completion of reaction was monitored by TLC. After completion of reaction, product (impurity-1) was extracted in DCM (50 mL). Obtained DCM layer was concentrated to get impurity-1 as white solid. Yield: 7.5 g (69.3%); MS m/z: 222 (M+1); ^{1}H NMR ($CDCl_3$): δ 7.88-7.84 (m, 2H), 7.77-7.73 (m, 2H), 4.01-3.81 (m, 3H), 3.70-3.58 (m, 2H), 2.73 (bs, 2H); ^{13}C NMR ($CDCl_3$): 169.19, 134.42, 131.95, 123.69, 70.40, 63.92, 40.59; IR: 3449.64 (-OH), 1769.90 (C=O), 1719.84 (C=O); Purity by HPLC: 96.37%.



Scheme-1: Synthesis of Rivaroxaban (**1**).

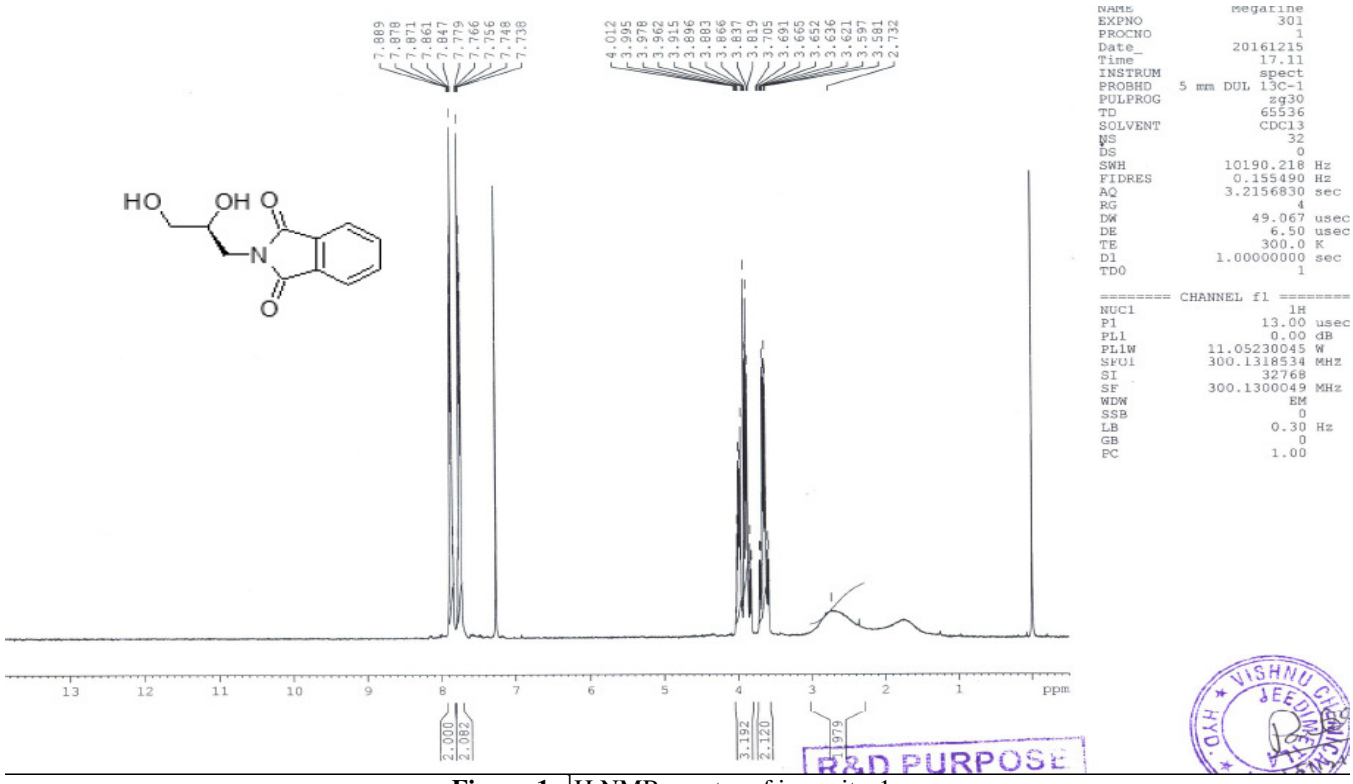


Figure-1: ^{1}H NMR spectra of impurity-1.

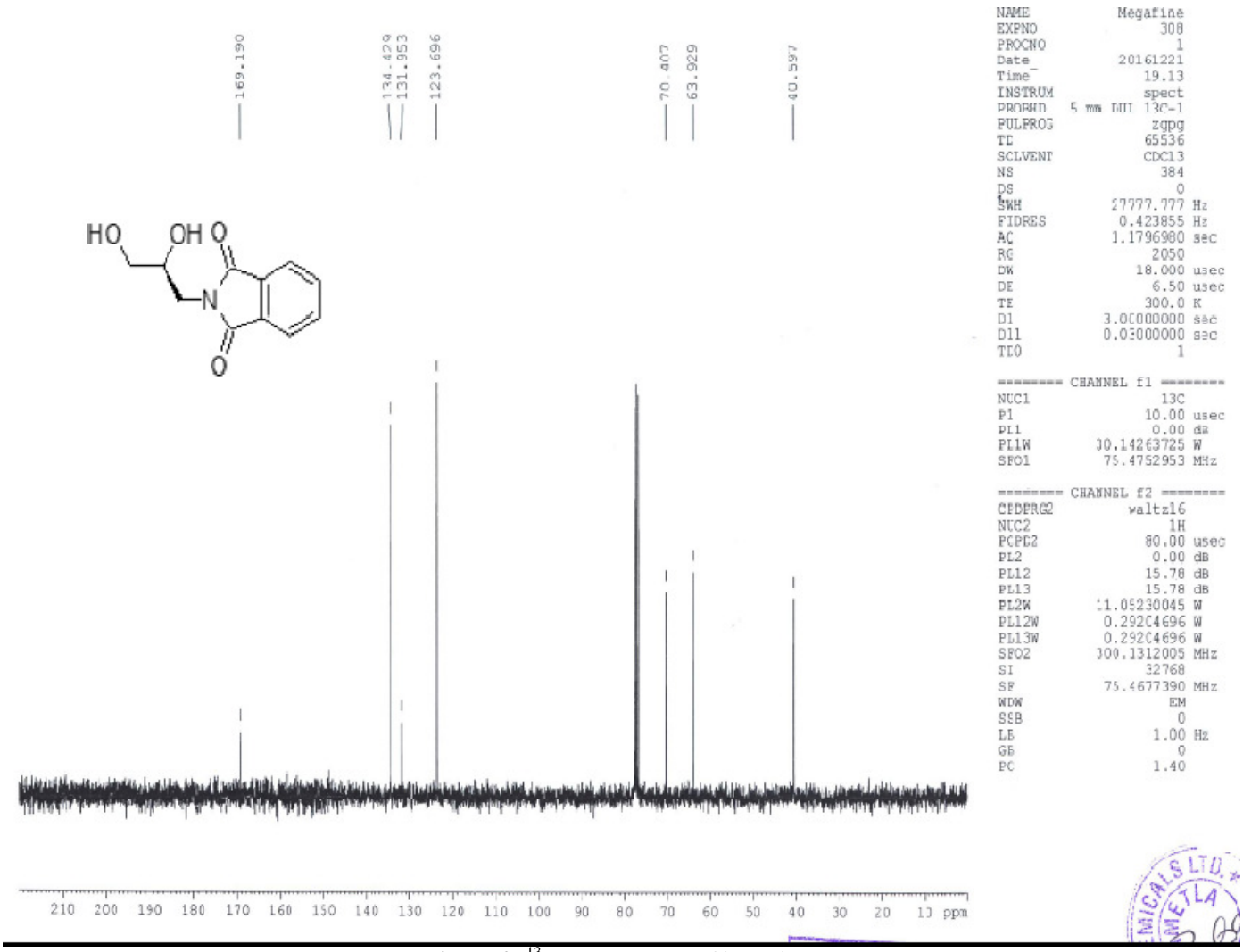


Figure-2: ^{13}C NMR spectra of impurity-1.

Synthesis of [2-({4-[(5S)-5-({[(5-chloro-2-thienyl) carbonyl] amino} methyl)-2-oxo-1,3-oxazolidin-3-yl]phenyl} amino) ethoxy] acetic acid (Impurity-2): To the solution of 4-{4-[(5S) -5-(aminomethyl)-2-oxo-1,3-oxazolidin-3-yl] phenyl} morpholin-3-one hydrochloride (6, 10 g, 0.030 mol) in water (10 mL) and DMSO (100 mL) was added conc. hydrochloric acid (30 mL) and heated to 75-80 °C for 5-6 h. Cooled the mass to 25-30 °C. The pH of reaction mass was adjusted to 7-8 using aqueous ammonia. Precipitated solid was filtered, washed with water (100 mL) and dried at 55-60 °C under vacuum for 5-6 h to obtain **8** as light brown colored solid**.** Yield: 8.0 g (84.3%). MS m/z: 310 (M+1); IR: 3175.35 (carboxylic acid –OH), 3536.46 and 3418.0 (Primary amine, NH_2), 3338.47 (secondary amine, NH), 1745.28 (acid, C=O), 1730.69 (carbamate, C=O), 1521.88 (aromatic, C=C) and 1088.52 (aliphatic ether, C-O-C); Purity by HPLC: 99.5%.

4-nitrophenyl 5-chlorothiophene-2-carboxylate (7, 7.3 g, 0.025 mol) and triethylamine (3.78 g, 0.0375 mol) was added to a solution of 8 (8.0 g, 0.025 mol) in DMSO (60 mL) at 35-40 °C. After completion of reaction was added water (300 mL) at 35-40°C. The pH of reaction mass was adjusted to 5-6 using conc. HCl and further stirred the reaction mass for 2 h at 25-30 °C. Precipitated solid was filtered, washed with water (100 mL), and dried under vacuum for 4-5 h at 50-55°C to get pure impurity-2 as light brown colored solid. Yield: 9.0 g (76.9%). MS m/z: 454 (M + 1). ^{1}H NMR (DMSO-*d6*): δ 9.21 (s, 1H), 7.81-7.80 (d, 1H), 7.20-7.19 (d, 3H), 6.55-6.53 (d, 2H), 6.47 (bs, 1H), 4.75-4.73 (m, 1H), 4.07-4.02 (m, 1H), 3.81-3.77 (m, 1H), 3.60-3.57 (m, 6H), 3.09-3.07 (bd, 2H). ^{13}C NMR (DMSO- *d6*): 173.77, 160.81, 154.48, 146.19, 138.68, 133.15, 128.77, 128.18, 126.99, 120.98, 111.85, 70.81, 68.56, 48.40; IR: 3330.07 (secondary amine, NH), 3092.43(carboxylic acid –OH), 1717.47 (carbamate, C=O), 1739.40 (acid, C=O), 1634.58 (secondary amide, C=O), 1525.43 (aromatic, C=C) and 1090.97; Purity by HPLC: 96.9%.

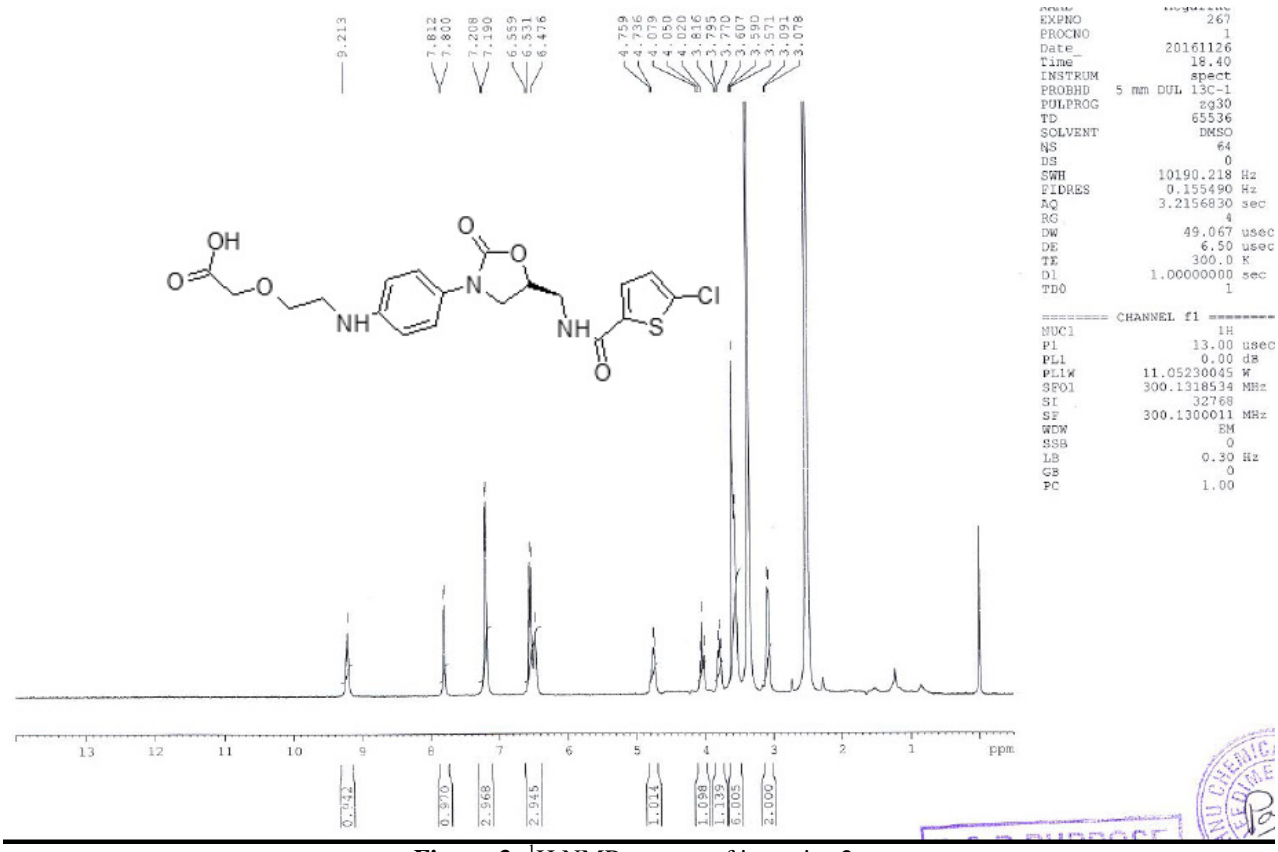


Figure-3: [1]H NMR spectra of impurity-2.

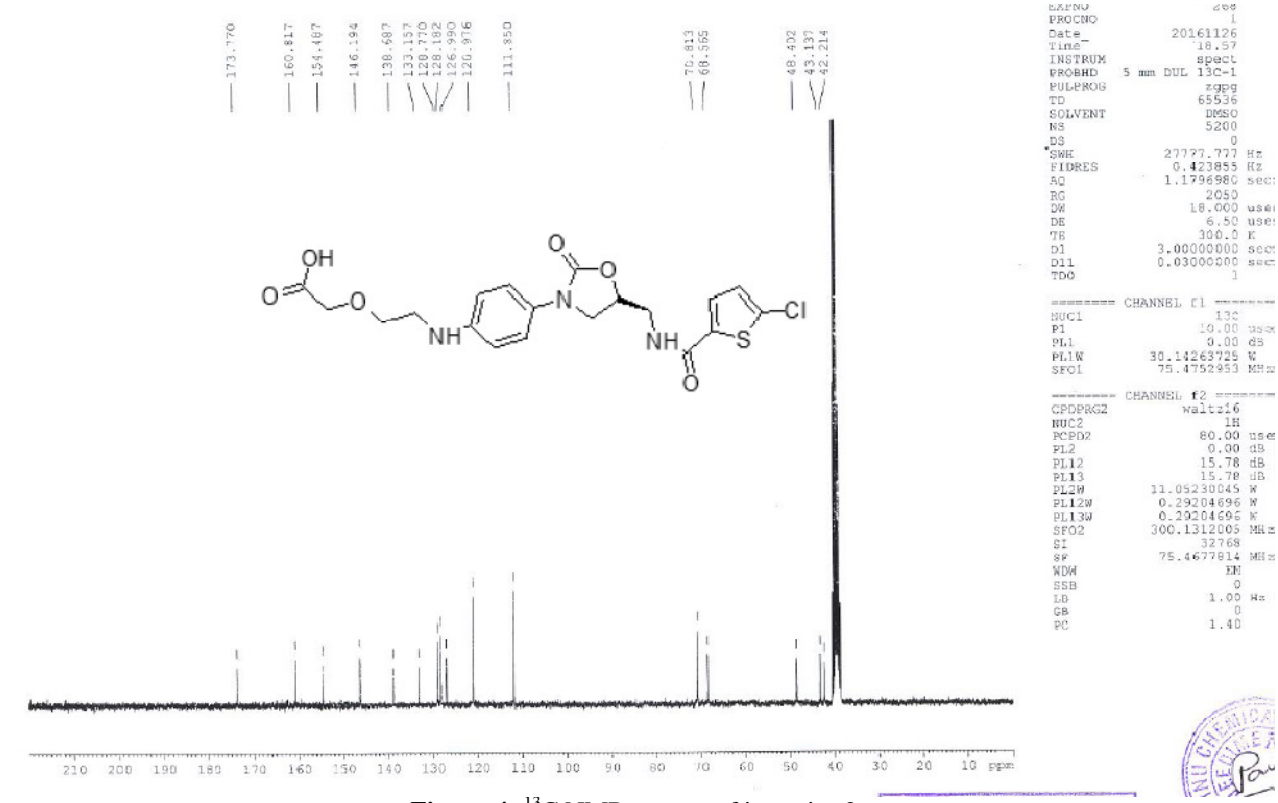


Figure-4: [13]C NMR spectra of impurity-2.

Preparation of 5-chloro-N-[(2R)-2-hydroxy-3-{[4-(3-oxomorpholin -4-yl)phenyl] amino} propyl] thiophene-2-carboxamide (Impurity-3): To the suspension of 2-[(2R)-2-hydroxy-3-{[4-(3-oxomorpholin-4-yl)phenyl]amino}propyl]-1H-isoindole-1,3(2H)-dione (**4**, 10 g, 0.025 mol) in methanol (100 mL) was added hydrazine hydrate (98% solution, 10 mL) and heated to reflux for 4 h. Mixture was cooled to 25-30 °C. Precipitated solid was filtered and washed with methanol (10 mL). Methanol filtrate was concentrated to get residue which was further degassed for 2 h at 60-65 °C under vacuum to obtain off white solid of **9**. **Yield**: 5.0 g (74.6 %). **MS m/z:** 266 (M+1). **^{1}H NMR (DMSO-*d6*):** δ 8.10(bs, 3H), 7.25-7.22 (d, 2H), 7.0-6.98 (d, 2H), 4.19 (s, 2H), 3.96-3.93 (t, 3H), 3.67-3.64 (t, 2H), 3.29-3.14 (m, 2H), 2.98 (s, 1H), 2.79-2.75 (m, 1H); **IR:** 3365.27 (-OH), 2953.08 (salt of Primary amine, RNH^+_3), 2887.11 (salt of secondary amine, NH), 1650.40 (amide, C=O), 1117.81 (ether), and 1605.49 (aromatic, C=C); **Purity by HPLC:** 99.5%;

4-nitrophenyl 5-chlorothiophene-2-carboxylate (**7**, 5.34 g, 0.018 mol) and triethylamine (2.7 g, 0.026 mol) was added to a solution of **9** (5.0 g, 0.018 mol) in DMSO (60 mL) at 35-40 °C. After completion of reaction was added water (300 mL) at 35-40°C. Reaction mixture was further cooled to 25-30 °C and stirred for 2 h. Precipitated solid was filtered, washed with water (100 mL), and dried under vacuum for 4-5 h at 50-55°C to get pure impurity-3 as white to off white solid. **Yield:** 6.0 g (77.7%). **MS m/z:** 410 (M+1). **^{1}H NMR (DMSO-*d6*):** δ 8.64-8.61 (t, 1H), 7.69-7.68 (d, 1H), 7.18-7.17 (d, 1H), 7.03-7.0 (d, 2H), 6.60-6.57 (d, 2H), 5.68-5.64 (t, 1H), 5.10-5.08 (d, 1H), 4.13 (s, 2H), 3.93-3.90 (t, 2H), 3.81-3.79 (t, 2H), 3.39 (m, 1H), 3.28-3.20 (m, 1H), 3.01-2.92 (m, 1H). **^{13}C NMR (DMSO- *d6*):** 165.77, 160.35, 147.45, 139.37, 132.17, 130.25, 128.03, 127.95, 126.48, 111.91, 67.78, 67.74, 63.56, 49.61, 47.38, 43.86; **IR:** 3365.78 (-OH), 1634.25 (cyclic amide, C=O), 1604.49 (secondary amide, C=O), 1519.59 (aromatic C=C), 997.31 (ether), and 1547.76 (secondary amine, NH); **Purity by HPLC:** 99.1%.

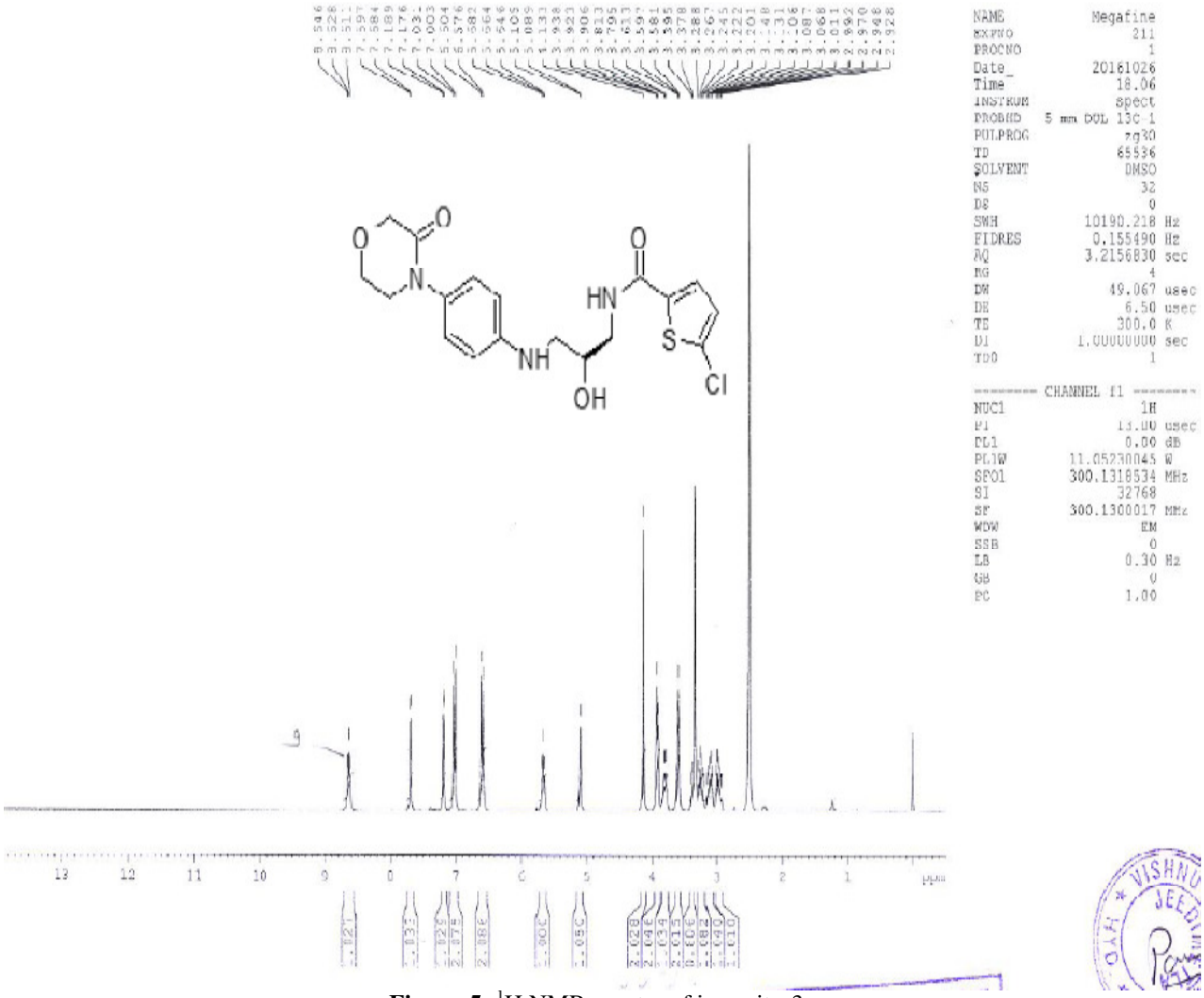


Figure-5: ^{1}H NMR spectra of impurity-3.

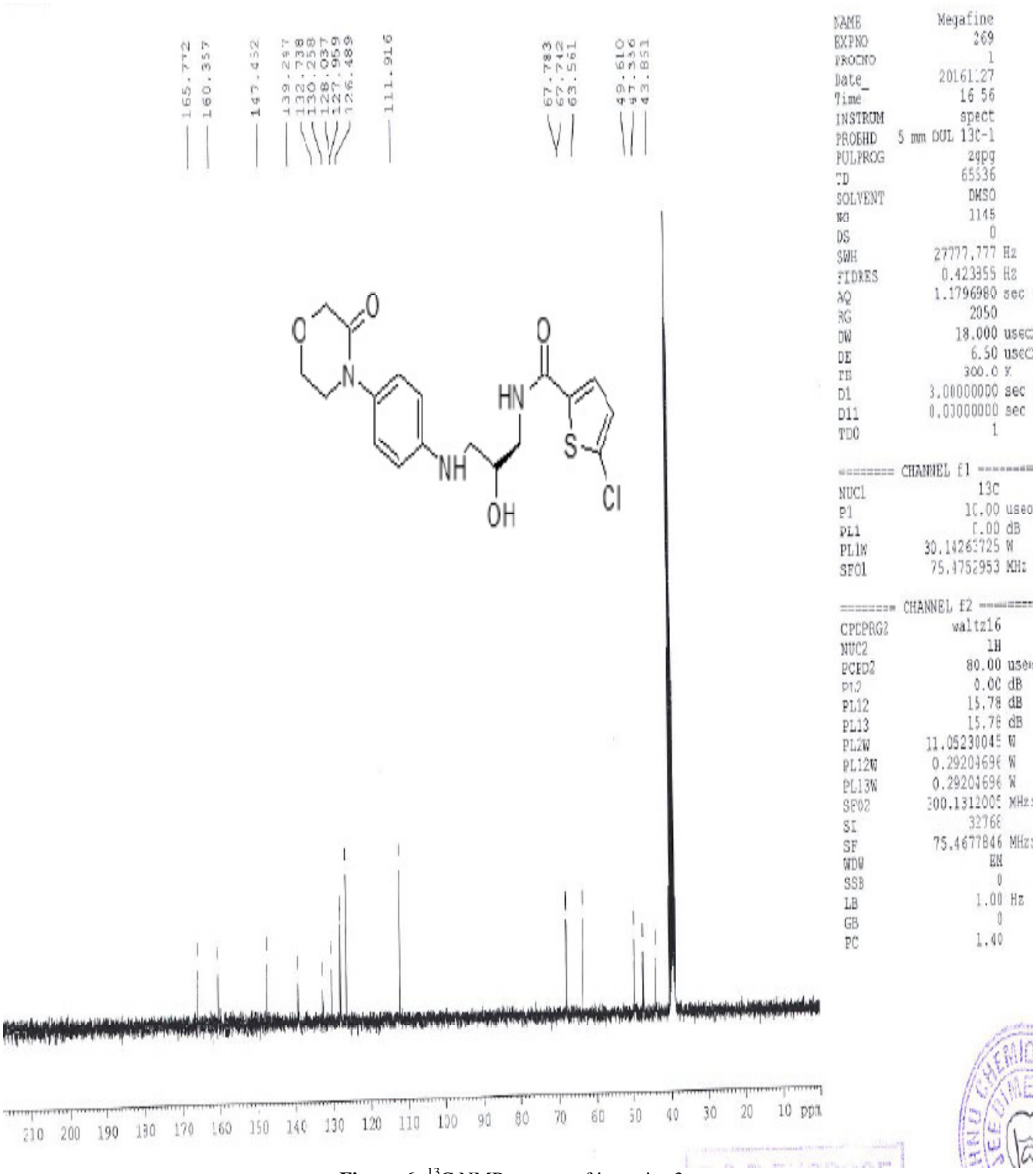


Figure-6: ^{13}C NMR spectra of impurity-3.

Results and Discussion

Rivaroxaban (**1**) prepared as per the literature process [4] and our recent process [8] (scheme-1) were subjected for LC-MS analysis to identify the relative molecular mass of impurities which were observed at 0.25, 0.37 and 0.81 RRT. Based on LC-MS analysis, the molecular mass of impurity-1, impurity-2 and Impurit-3 are found to 221, 453 and 409 respectively. According to LC-MS data and considering route of synthesis, following structures of impurities were envisaged (Figure-7).

Synthesis of Impurity 1: Impurity-1 was synthesized by opening epoxide ring of the readily available starting oxiran **6** (scheme-2). Oxiran (**6**) was treated with 10% aqueous sodium hydroxide solution in acetonitrile at elevated temperature. After completion of the reaction, reaction mixture was cooled and the product (impurity-1) was extracted into dichloromethane (DCM). The DCM layers were combined and concentrated to get white solid powder of impurity-1 in good yield and purity by HPLC.

Synthesis of Impurity 2: Impurity-2 was synthesized by following the synthetic Scheme-3, where amino-morpholinone (**6**) was treated with hydrochloric acid in mixture of water and dimethyl sulfoxide (DMSO) at 80-85°C which results acid catalyzed opening of morpholinone ring of **6**. Neutralization of reaction mass using aqueous ammonia resulted precipitation of solid which was filtered and dried to get light brown color powder of amino-acid intermediate **8**. Primary amine of obtained **8** was selectively coupled with active ester (**7**) in the presence of triethyl amine (TEA) in DMSO. After completion of reaction, methanol was added to the reaction mass and neutralized with hydrochloric acid to provide light brown color solid of impurity-2 with good yield and HPLC purity.

Synthesis of Impurity 3: Synthesis of impurity-3 was started from **4** (Scheme-4) where phthalamide group was deprotected using hydrazine hydrate in methanol at elevated temperature. After completion of reaction, precipitated by-product was filtered at room temperature to obtain clear filtrate containing free base of **9.** The obtained methanol layer of **9** was acidified with conc. HCl to provide white solid of **9**. The obtained **9** was selectively coupled with active ester (**7**) in the presence of triethyl amine in DMSO at elevated temperature. After completion of reaction water was added as an anti-solvent to obtained white solid of impurity-3 with good yield and HPLC purity.

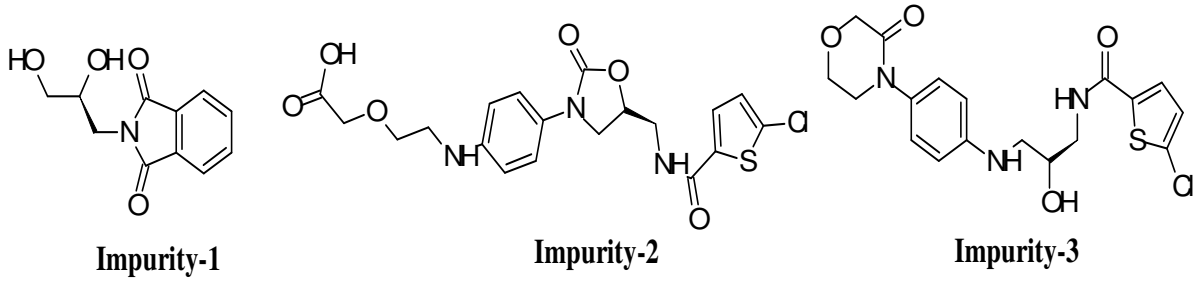
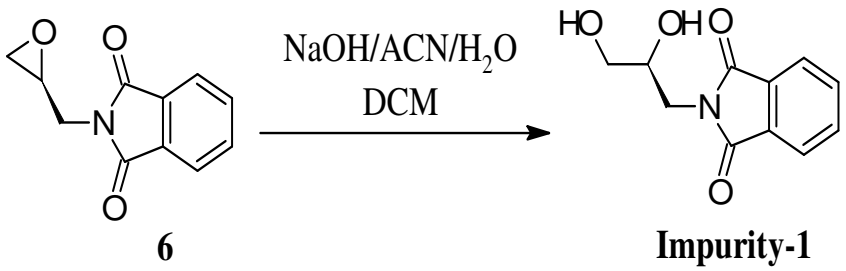
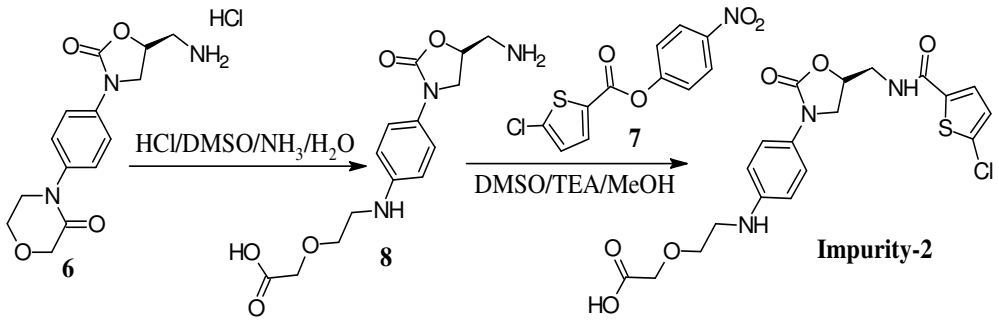


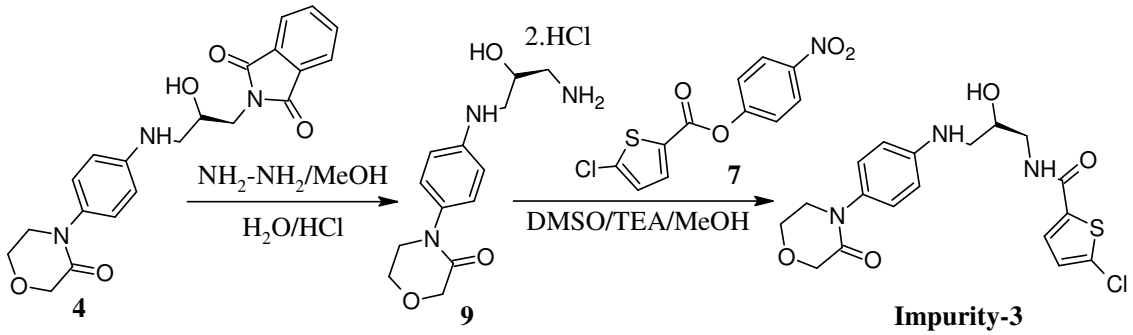
Figure-7: proposed structure for impurity-1, 2 and 3.



Scheme-2: Synthesis of impurity-1.



Scheme-3: Synthesis of impurity-2.



Scheme-4: Synthesis of impurity-3.

Conclusion

Related substances (impurity-1, 2 and 3) generated during manufacturing of Rivaroxaban (**1**) were identified, synthesized and characterized. Practical, high yielding and operationally simple approach for the synthesis of impurities is developed. We believe that developed synthetic approach will be immensely useful to the chemists working in the field of process as well as analytical research.

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