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Research Article

Synthesis and Characterization of Tolvaptan Impurities

Madhuresh Kumar Sethi, Vijendra Singh Rawat, Jayaprakash Thirunavukarasu, Rajakrishna Yerramalla, and Anish Kumar

R and D, Mylan Laboratories Ltd., Plot No. 31, 32, 33 and 34 A ANRICH Industrial Estate, Bollaram, Jinnaram, Medak, Andhra Pradesh 502325, India

Correspondence should be addressed to Madhuresh Kumar Sethi; madhuresh.sethi@mylan.in

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Twenty-six possible as well as observed impurities during the preparation of Tolvaptan have been identified, prepared, and characterized by HPLC (high performance liquid chromatography), NMR (nuclear magnetic resonance), and mass spectra. Control of these impurities, formed during various stages of Tolvaptan preparation, has been mentioned in this paper.

1. Introduction

Impurity profiling is a major step in the drug development process followed by drug making companies world over [1, 2]. Impurities even if present in fairly small quantity could affect the overall safety and efficacy of the drug. Hence it is of tremendous importance to prepare an impurity profiling of the drug and to set their limits within the range set by ICH [3]. Drug related impurities could be classified into categories such as starting material, intermediate, degradation products, by-products derived from impurities in starting material and by-product stability, and shelf life derived from side reaction. There are several reports wherein impurities related to drug are first identified and then prepared and characterized by different methods such as MASS, NMR, and HPLC [4].

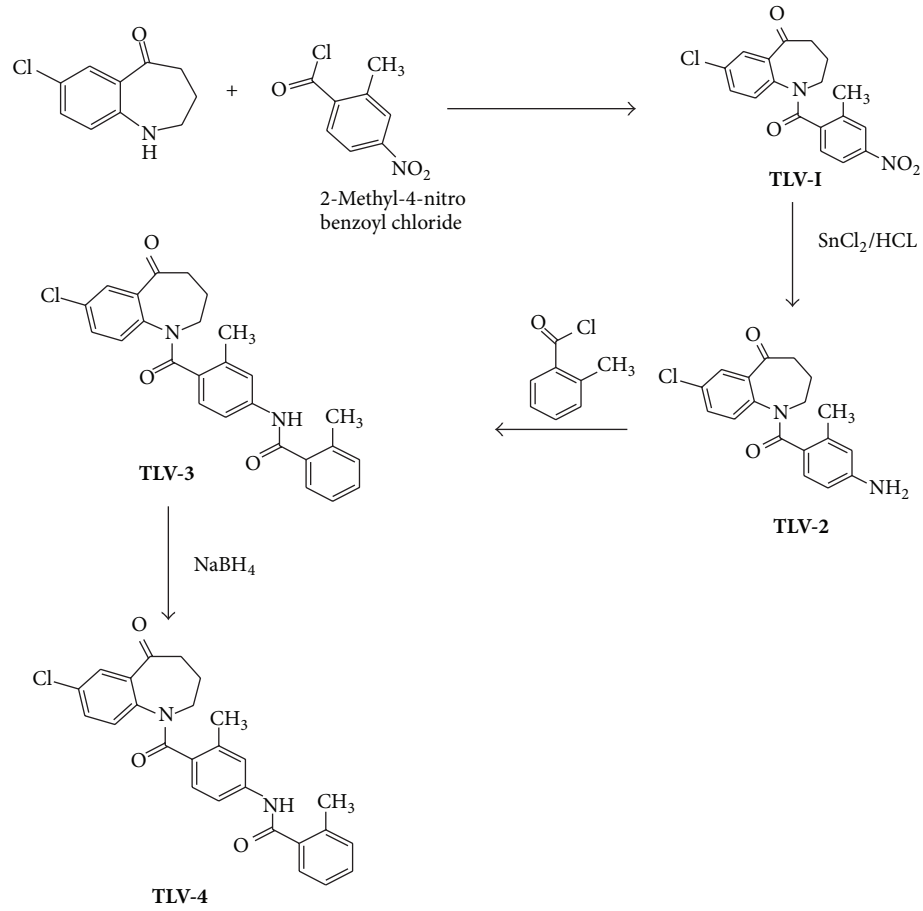
Tolvaptan (N-[±-4-[(7-chloro-2,3,4,5-tetrahydro-5-hydroxy-1H-1-benzazepin-1-yl) carbonyl]-3-methylphenyl]-2-methyl-benzamide) is a drug used to treat hyponatremia associated with congestive heart failure, cirrhosis, and the syndrome of inappropriate antidiuretic hormone [5, 6]. It is available under the brand name Samsca produced by Otsuka Pharmaceutical Co Ltd. Tolvaptan is metabolized by the CYP3A4 in the liver.

Various methods for synthesis of Tolvaptan have been reported in the literature [7–11]. During the preparation of Tolvaptan, impurities were detected in HPLC. Process used to prepare Tolvaptan involves condensing 7-chloro-1, 2, 3, 4-tetrahydro-benzo[b]azepin-5-one with 2-methyl, 4-nitro benzoyl chloride, followed by reduction using $SnCl_2$/HCl catalyst resulting in amine which is then condensed with o-toluoyl chloride followed by reduction with sodium borohydride to give Tolvaptan pharma (Scheme 1).

A comprehensive study was undertaken to synthesize and characterize these impurities by spectroscopic techniques. Present studies describe the synthesis and characterization of possible as well as observed impurities in the process for preparation of Tolvaptan. As per the guidelines recommended by ICH (the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use), the acceptable level for a known or unknown related compound (impurity) is less than 0.15 and 0.10%, respectively, in a drug substance. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified and characterized. Present work deals with the identification, synthesis, and characterization of impurities/related substances of Tolvaptan.

2. Experimental

2.1. Materials and Methods. All the chemicals are commercially available and used without purification.The ^{1}H NMR was recorded in DMSO at 300 MHz on a Bruker 300 MHz Fourier transform NMR spectrometer. The chemical shifts were reported in δ ppm relative to TMS. Mass spectrum was recorded on Agilent 1100 Series LC-MSD trap-SL system.



Scheme 1: Synthetic route used for preparation of Tolvaptan.

2.2. Synthetic Procedures

2.2.1. Preparation of Tolvaptan Impurity A. To a solution of 7-chloro-1,2,3,4-tetrahydro-benzo[b]azepin-5-one (5 g, 0.0303 mol) at 0°C in 40 mL methylene dichloride triethyl amine (10.1 mL, 0.0727 mol) was added dropwise. Reaction mixture was stirred at room temperature for 15 minutes. *o*-Toluoyl chloride (4.174 mL, 0.0302 mol) was charged and the resulting reaction mixture was stirred for 2 h. Reaction mixture was washed with demineralized water (40 mL) and then with dilute hydrochloric acid (40 mL, 10%). Organic layer was separated and dried over sodium sulfate and distilled under vacuum to get product as light yellow solid (Scheme 2).

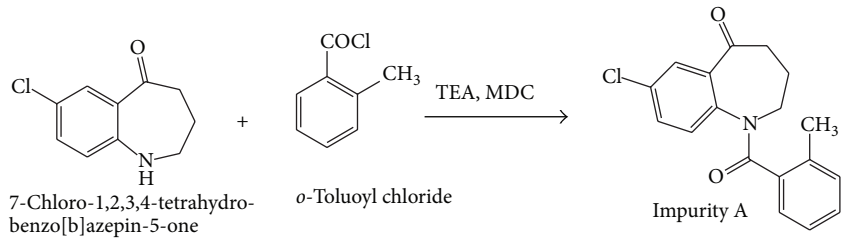
(5.5 g, Yield = 58%). HPLC purity = 99.33%, MS *m/z* = 314.17 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 7.56–6.93 (7H, *m*), 3.6–3.3 (2H, *m*), 2.3 (3H, *s*), 2.1–1.9 (2H, *m*), 1.04–1.02 (2H, *m*).

2.2.2. Preparation of Tolvaptan Impurity B. To a solution of A (3 g, 0.00958 mol) at 0°C in 30 mL methanol sodium borohydride (0.181 g, 0.004 mol) was added portionwise. Reaction mixture was stirred for half an hour at the same temperature. Solvent of the reaction mixture distilled under vacuum. 40 mL MDC was added to the residue. It was then washed with water (40 mL) and with dil. HCl (40 mL, 10%). Organic layer was separated and dried over sodium sulfate and distilled under vacuum to get product as white solid (Scheme 3).

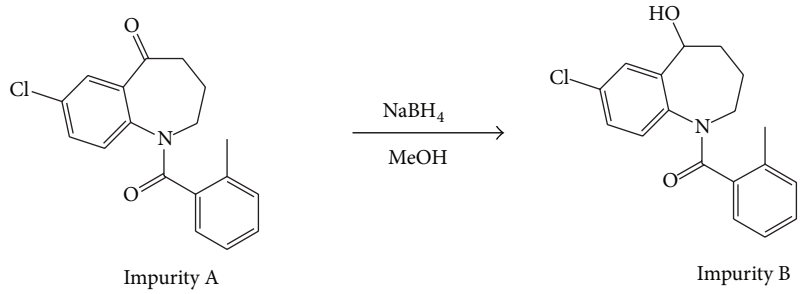
(2.5 g, % Yield = 83%). HPLC purity = 96.87%, MS *m/z* = 316.19 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 7.57–6.93 (7H, *m*), 4.0 (1H, *m*), 3.6–3.3 (3H, *m*), 2.4 (3H, *s*), 2.1–1.9 (2H, *m*), 1.04–1.02 (2H, *m*).

2.2.3. Preparation of Tolvaptan Impurity C. To a solution of 2-methyl 4-amino benzoic acid (5 g, 0.0331 mol) in MDC (40 mL) at 0°C triethyl amine (10.1 mL, 0.0727 mol) was added dropwise. Reaction mixture was stirred at room temperature for 15 minutes. *o*-Toluoyl chloride (4.320 mL, 0.0330 mol) was added to the reaction mixture. Reaction mixture was stirred for 2 hours. RM was washed with DM water (40 mL) and with dilute HCl (40 mL, 10%). Organic layer was separated and dried over sodium sulfate. Methanol was also added to dissolve the undissolved particles. Solvent distilled under vacuum to get the crude as light yellow solid product which is then washed with ethyl acetate (30 mL) to get product as white solid (Scheme 4).

(4 g, % Yield = 45%) HPLC purity = 95.71%, MS *m/z* = 270.19 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 12.5



Scheme 2: Synthetic route for Impurity A.



Scheme 3: Synthetic route for Impurity B.

(1H, *s*), 10.3 (1H, *s*), 7.87–7.84 (1H, *m*), 7.68–7.65 (2H, *m*), 7.47–7.38 (2H, *m*), 7.33–7.28 (2H, *m*), 2.52–2.49 (3H, *m*), 2.38 (3H, *s*).

2.2.4. Preparation of Tolvaptan Impurity D. To a solution of compound with formula C (3 g, 0.0091 mol) in methanol (40 mL) at 0°C sodium borohydride (0.3 g, 0.000126 mol) was added portionwise. The reaction mixture was stirred at the same temperature for 0.5 h. Solvent of the reaction mixture distilled under vacuum. MDC was added to the residue (40 mL). The RM was washed with water (40 mL) and with dil. HCl (40 mL, 10%). Organic layer was separated and dried over sodium sulfate and distilled under vacuum to get product as light yellow solid (Scheme 5).

(2.5 g, % Yield = 83%) HPLC purity = 91.66%, MS *m/z* = 331.0 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.44–1.77 (2H, *m*), 1.94–2.11 (2H, *m*), 2.19 (3H, *s*), 2.65–2.73 (2H, *m*), 4.80–4.92 (1H, *m*), 5.30 (2H, *m*), 5.72 (1H, *d, J* = 7 Hz), 6.10–7.6 (6H, *m*).

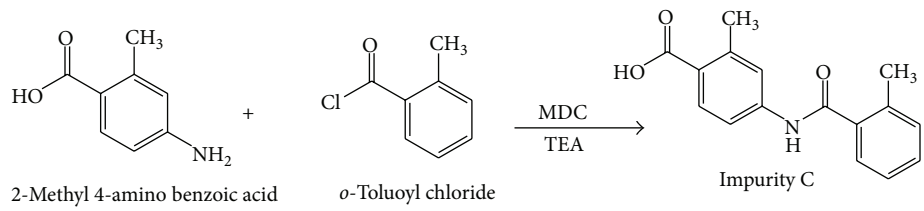
2.2.5. Preparation of Tolvaptan Impurity E. To a solution of Tolvaptan pharma (2 g, 0.00447 mol) in MDC (40 mL) o-toluoyl chloride (2.4 mL, 0.0200 mol) was added. Reaction mixture was stirred for 24 hours. Solvent distilled under vacuum to get the crude as light yellow solid product purified by column chromatography (Hexane: Ethyl acetate) (Scheme 6).

(800 mg, % Yield = 32%) HPLC purity = 91.5%, MS *m/z* = 567.20 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.46–1.75 (2H, *m*), 1.93–2.02 (1H, *m*), 2.05–2.12 (1H, *m*), 2.64–2.73 (1H, *m*), 2.26 (6H, *s*), 2.30 (3H, *s*), 4.83–4.87 (1H, *m*), 5.70 (1H, *s*), 6.59 (1H, *d*), 6.79–6.96 (2H, *d, J* = 7.2 Hz), 7.11–7.42 (8H, *m*), 7.54–7.66 (2H, *m*).

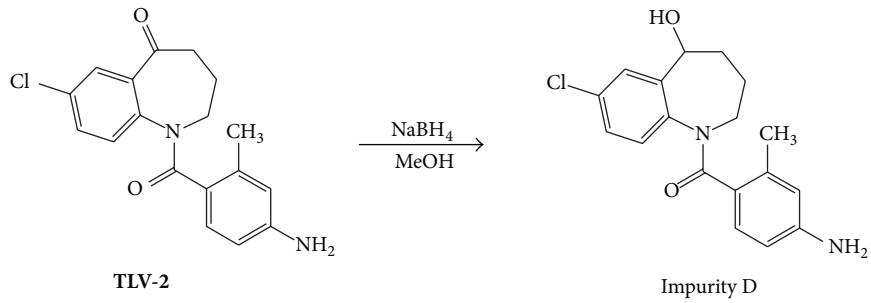
2.2.6. Preparation of Tolvaptan Impurity F. To a solution of 4-nitro benzoic acid (11 g, 0.0658 mol) in toluene (100 mL) and *N*, *N* dimethylformamide (3 mL) at 0°C thionyl chloride (7.2 mL, 0.0949 mol) was added dropwise. Reaction mixture was heated 60°C for 45 minutes. Solvent and excess thionyl chloride was distilled under reduced pressure. To a solution of 7-chloro-1, 2, 3, 4-tetrahydro-benzo[b]azepin-5-one (9.78 g, 0.0592 mol) in MDC (100 mL), triethyl amine (27 mL, 0.197 mol) was added and stirred for 15 minutes. The isolated acid chloride of 4-nitro benzoic acid was added to the reaction mixture and stirred for 2 h. RM was washed with DM water (100 mL) and with dilute HCl (100 mL, 10%). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 7).

(Yield = 15 g, % Yield = 66%). HPLC purity = 91.34%, MS *m/z* = 345.0 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 8.5–8.4 (2H, *m*), 8.4–8.2 (2H, *m*), 7.6–7.4 (3H, *m*), 3.0–2.5 (4H, *m*), 1.3–1.0 (2H, *m*).

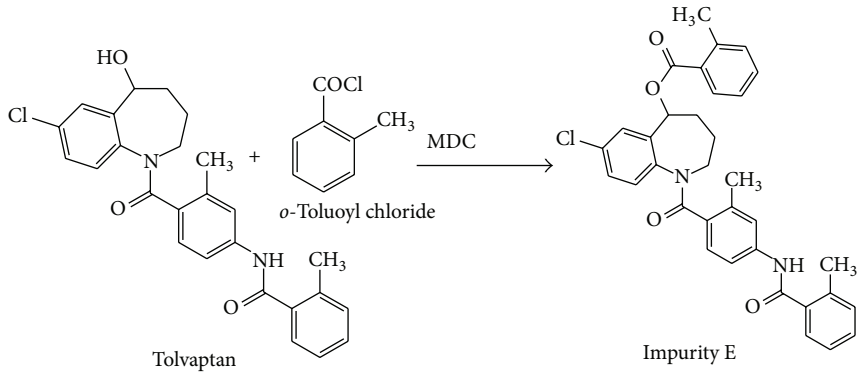
2.2.7. Preparation of Tolvaptan Impurity G. To a solution of F (7 g, 0.0203 mol) in ethanol (50 mL) $SnCl_2$ (13.7 g, 0.0610 mol) was added. Reaction mixture cooled to 0°C and conc. HCl (23 mL) was added dropwise. RM was stirred at room temperature for 16 hours. Reaction mixture was poured into crushed ice (100 mL). pH of the solution was adjusted to 9 using sodium hydroxide solution. Ethyl acetate (100 mL) was added to it and stirred for 30 minutes. Organic layer was separated, dried over sodium sulfate, and concentrated under vacuum to get product as light yellow solid (Scheme 8). (6.1 g, % Yield = 95%). HPLC purity = 90.9%, MS *m/z* = 315.0 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 8.4–8.0 (2H, *m*), 7.6–7.2 (2H, *m*), 7.0–6.8 (3H, *m*), 5.2 (2H, *s*), 3.0–2.5 (4H, *m*), 1.3–1.0 (2H, *m*).



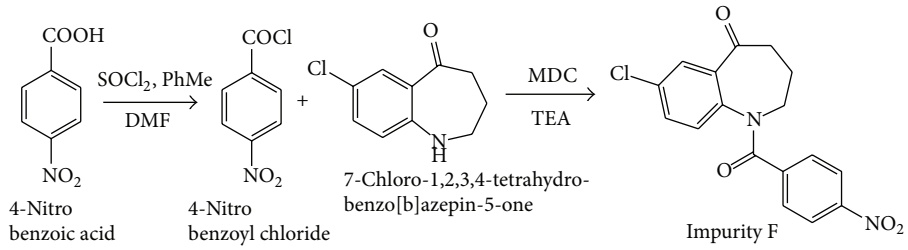
Scheme 4: Synthetic route for Impurity C.



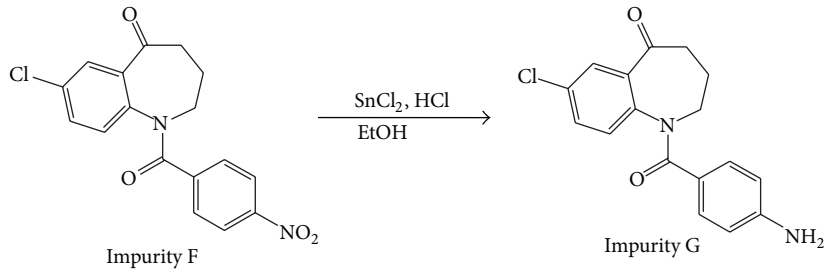
Scheme 5: Synthetic route for Impurity D.



Scheme 6: Synthetic route for impurity E.



Scheme 7: Synthetic route for Impurity F.



Scheme 8: Synthetic route for Impurity G.

2.2.8. Preparation of Tolvaptan Impurity H. To a solution of G (5 g, 0.00318 mol) in MDC (40 mL) at 0°C triethyl amine (6.6 mL, 0.0473 mol) was added dropwise. Reaction mixture was stirred at room temperature for 45 minutes. *o*-Toluoyl chloride (2.5 mL, 0.0191 mol) was added to the reaction mixture and stirred for 1 h. Reaction mixture was washed with DM water (40 mL) and with dilute HCl (40 mL, 10%). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 9).

(6 g, % Yield = 87%). HPLC purity = 92.94%, MS *m/z* = 433.0 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 10.5 (1H, *s*), 7.7-7.6 (3H, *m*), 7.4-7.3 (3H, *m*), 7.2-7.1 (4H, *m*), 6.9-6.8 (1H, *m*), 4.5–4.0 (1H, *m*), 2.7 (1H, *s*), 2.4 (3H, *s*), 2.0-1.9 (2H, *m*), 1.3-1.2 (1H, *m*).

2.2.9. Preparation of Tolvaptan Impurity I. To a solution of H (4 g, 0.009 mol) in methanol (40 mL) at 0°C sodium borohydride (0.175 g, 0.00462) was added portionwise. The reaction mixture was stirred at the same temperature for 0.5 h. Solvent of the reaction mixture was distilled under vacuum. 40 ml MDC was added to the residue. It was washed with water (40 mL) and with dil. HCl (40 mL). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as white solid (Scheme 10).

(2.5 g, % Yield = 62%). HPLC purity = 94.37%, MS *m/z* = 435.1 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 10.4 (1H, *s*), 7.6-7.5 (3H, *m*), 7.4-7.3 (3H, *m*), 7.1-7.0 (4H, *m*), 6.9-6.8 (1H, *m*), 4.5–4.0 (1H, *m*), 3.8–3.6 (1H, *m*), 3.4-3.3 (1H, *m*), 2.7 (1H, *s*), 2.4 (3H, *s*), 2.0-1.9 (2H, *m*), 1.3-1.2 (1H, *m*).

2.2.10. Preparation of Tolvaptan Impurity J. To a solution of 4-methyl benzoic acid (3 g, 0.0220 mol) in toluene (20 mL) and DMF (0.2 mL) at 0°C thionyl chloride (2.5 mL, 0.0210 mol) was added dropwise. Reaction mixture was heated 60°C for 30 minutes. Solvent and excess thionyl chloride was distilled under reduced pressure. In another reaction, to a solution of **TLV-2** (5 g, 0.0152 mol) MDC (20 mL) triethyl amine (6 mL) was added. Isolated acid chloride of 4-methyl benzoic acid was added to the reaction mixture. Reaction mixture was stirred for 45 minutes. RM was washed with DM water (30 mL) and with dilute HCl (30 mL, 10%). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 11).

(6 g, % Yield = 61%). HPLC purity = 81.66%, which is further purified by flash column chromatography. MS *m/z* = 447.1 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.99 (2H, *m*), 2.30 (3H, *s*), 2.35 (3H, *s*), 2.80 (2H, *m*), 3.71 (2H, *m*), 7.25–7.44 (7H, *m*), 7.61–7.65 (1H, *m*), 10.29 (1H, *s*).

2.2.11. Preparation of Tolvaptan Impurity K. To a solution of J (1.5 g, 0.0033 mol) in methanol (20 mL) at 0°C sodium borohydride (0.38 g, 0.0100 mol) was added portionwise. Reaction mixture was stirred at the same temperature for 0.5 h. Solvent of the reaction mixture was distilled under vacuum. MDC was added to the residue (30 mL) and then washed with water (30 mL) and with dil. HCl (30 mL). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as white solid (Scheme 12).

(900 mg, % Yield = 60%). HPLC purity = 84.61%, MS *m/z* = 449.1 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.95 (2H, *m*), 2.30 (3H, *s*), 2.35 (3H, *s*), 2.80 (2H, *m*), 3.71–3.95 (3H, *m*), 5.41 (1H, *d, J* = 7.1 Hz), 7.25–7.44 (7H, *m*), 7.61–7.65 (1H, *m*), 10.29 (1H, *s*).

2.2.12. Preparation of Tolvaptan Impurity L. To a solution of 3-methyl benzoic acid (2 g, 0.0146 mol) in toluene (20 mL) and DMF (0.2 mL) at 0°C thionyl chloride (1.7 mL, 0.0142 mol) was added dropwise. Reaction mixture was heated to 60°C for 30 minutes. Solvent and excess thionyl chloride was distilled under reduced pressure. In another reaction, to solution of **TLV-2** (5 g, 0.0152 mol) in MDC (20 mL) triethyl amine (5 mL) was added and stirred for 15 minutes. Isolated acid chloride of 3-methyl benzoic acid was added to the reaction mixture and stirred for 45 minutes. RM was washed with DM water (30 mL) and with dilute HCl (30 mL, 10%). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 13).

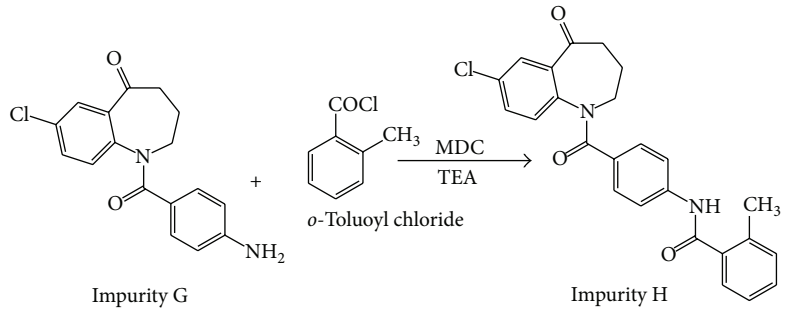
(4 g, % Yield = 61%). HPLC purity = 79.2% which is further purified by flash column chromatography, MS *m/z* = 447.1 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.99 (2H, *m*), 2.30 (3H, *s*), 2.35 (3H, *s*), 2.80 (2H, *m*), 3.71 (2H, *m*), 7.25–7.44 (7H, *m*), 7.51–7.60 (1H, *m*), 10.32 (1H, *s*).

2.2.13. Preparation of Tolvaptan Impurity M. To a solution of L (1.5 g, 0.0033 mol) in methanol (20 mL) at 0°C sodium borohydride (0.38 g, 0.0102 mol) was added portionwise. Solvent of the reaction mixture was distilled under vacuum. MDC (40 mL) was added to the residue and washed with water (40 mL) and with dil. HCl (40 mL). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 14).

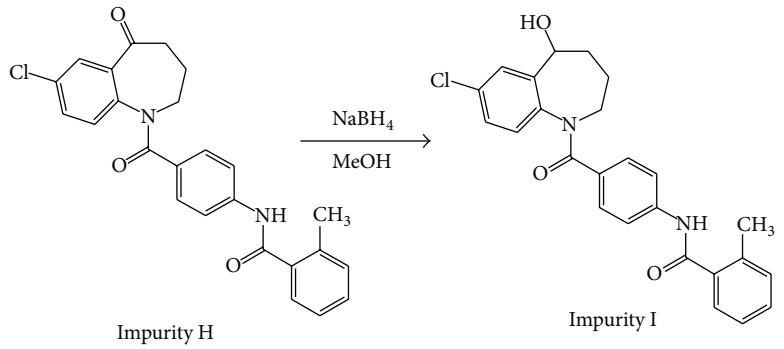
(1.0 g, % Yield = 66%), MS *m/z* = 449.1 (M + H); ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.98 (2H, *m*), 2.28 (3H, *s*), 2.35 (3H, *s*), 2.80 (2H, *m*), 3.71–3.95 (3H, *m)*, 5.41 (1H, *d, J* = 7.1 Hz*)*, 7.28–7.44 (7H, *m*), 7.60–7.65 (1H, *m*), 10.32 (1H, *s*).

2.2.14. Preparation of Tolvaptan Impurity N. To a solution of 3,4-dimethyl benzoic acid (3.5 g, 0.0233 mol) in toluene (30 mL) and DMF (1 mL) at 0°C thionyl chloride (4.1 mL, 0.0344 mol) was added dropwise. Reaction mixture was heated to 60°C for 45 minutes. Solvent and excess thionyl chloride was distilled under reduced pressure. In another reaction, to a solution **TLV-2** (5 g, 0.0152 mol) in MDC (30 mL) triethyl amine (6 mL, 0.0810 mol) was added. The reaction mixture was stirred for 15 minutes. The isolated acid chloride of 3,4-dimethyl benzoic acid was added to the reaction mixture. Reaction mixture stirred for 45 minutes at room temperature. RM was washed with DM water (30 mL) and with dilute HCl (30 mL, 10%) Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as yellow solid (Scheme 15).

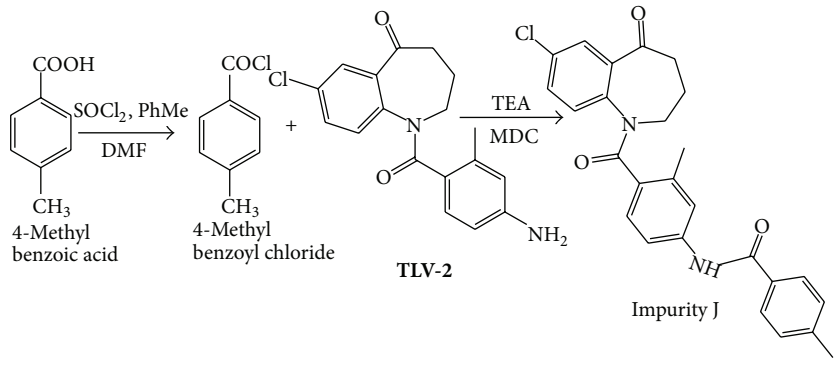
(7 g, % Yield = 65%). HPLC purity = 71.5%, which is further purified by flash column chromatography, MS *m/z* = 461.1 (M + H); ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 2.00 (2H, *m*), 2.30 (3H, *s*), 2.35 (3H, *s*), 2.40 (3H, *s*), 2.85 (2H, *m*),



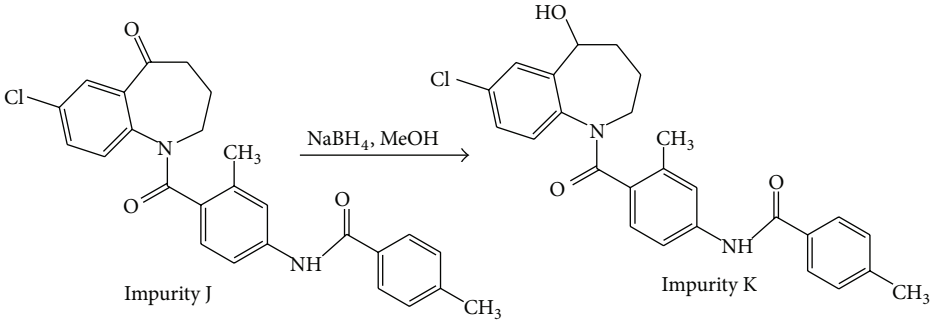
Scheme 9: Synthetic route for Impurity H.



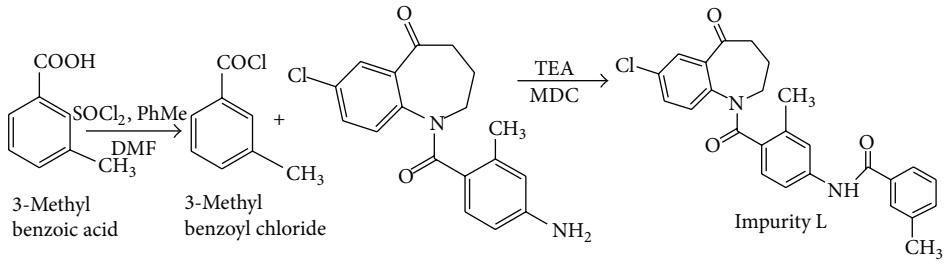
Scheme 10: Synthetic route for Impurity I.



Scheme 11: Synthetic route for Impurity J.

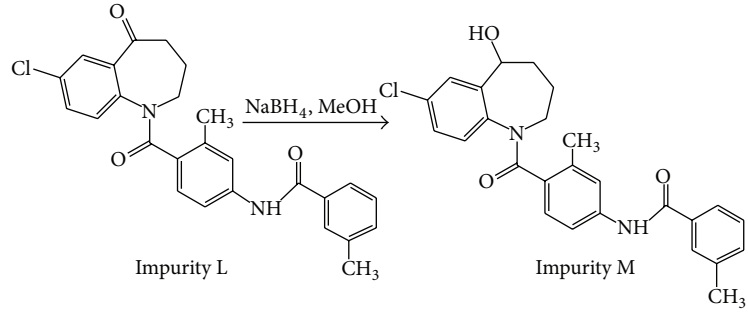


Scheme 12: Synthetic route for Impurity K.



TLV-2

Scheme 13: Synthetic route for Impurity L.



Scheme 14: Synthetic route for Impurity M.

3.78 (2H, *m*), 7.25–7.44 (6H, *m*), 7.51–7.60 (1H, *m*), 10.28 (1H, *s*).

2.2.15. Preparation of Tolvaptan Impurity O. To a solution of N (1.5 g, 0.0033 mol) in methanol (20 mL) at 0°C sodium borohydride (0.38 g, 0.00978 mol) was added portionwise. Solvent of the reaction mixture was distilled under vacuum. MDC was added to the residue (30 mL) and washed with water (30 mL) and with dil. HCl (30 mL). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 16).

(800 mg, % Yield = 53%), MS *m/z* = 463.1 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 2.00 (2H, *m*), 2.30 (3H, *s*), 2.35 (3H, *s*), 2.40 (3H, *s*), 2.85 (3H, *m*), 3.78 (2H, *m*), 5.41 (1H, *d, J* = 7.3 Hz), 7.25–7.44 (6H, *m*), 7.51–7.60 (1H, *m*), 10.25 (1H, *s*).

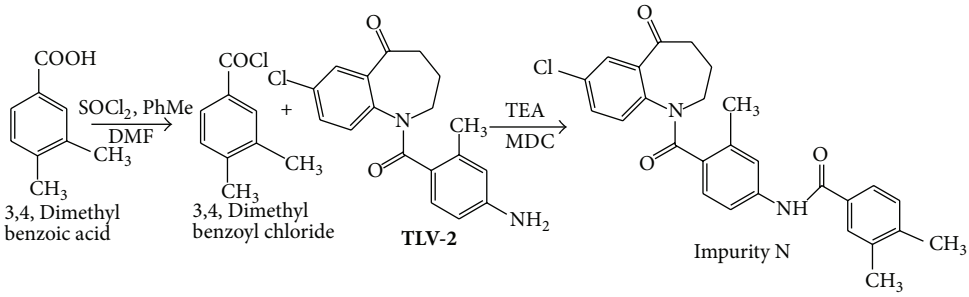
2.2.16. Preparation of Tolvaptan Impurity P. To a solution of 2,4-dimethyl benzoic acid (3.0 g, 0.0199 mol) in toluene (30 mL) and DMF (1 mL) at 0°C thionyl chloride (3.2 mL, 0.0294 mol) was added dropwise. Reaction mixture was heated 60°C for 45 minutes. Solvent and excess thionyl chloride was distilled under reduced pressure. In another reaction, to a solution of **TLV-2** (4 g, 0.0121 mol) in MDC (30 mL) triethyl amine (6 mL, 0.0810 mol) was added. Isolated acid chloride of 2,4-dimethyl benzoic acid was added to the reaction mixture and stirred for 45 minutes. RM was washed with DM water (30 mL) and with dilute HCl (30 mL, 10%). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as yellow solid (Scheme 17).

(5 g, %Yield = 54%). HPLC purity = 78.3% which is further purified by flash column chromatography, MS *m/z* = 461.1 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 2.05 (2H, *m*), 2.32 (3H, *s*), 2.35 (3H, *s*), 2.40 (3H, *s*), 2.85 (2H, *m*), 3.78 (2H, *m*), 7.25–7.48 (6H, *m*), 7.50–7.65 (1H, *m*), 10.29 (1H, *s*).

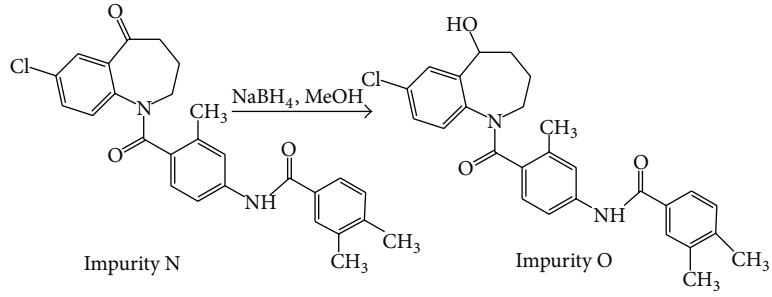
2.2.17. Preparation of Tolvaptan Impurity Q. To a solution of P (2.2 g, 0.00478 mol) in methanol (30 mL) at 0°C sodium borohydride (0.542 g, 0.01432 mol) was added portionwise. Reaction mixture was stirred for 0.5 h. Solvent of the reaction mixture distilled under vacuum. MDC was added to the residue (20 mL) and washed with water (20 mL) and with dil. HCl (20 mL). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 18).

(1.2 g, % Yield = 54%). HPLC purity = 88.77%, MS *m/z* = 463.1 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 2.10 (2H, *m*), 2.30 (3H, *s*), 2.35 (3H, *s*), 2.40 (3H, *s*), 2.87 (3H, *m*), 3.78 (2H, *m*), 5.42 (1H, *d, J* = 7.1 Hz), 7.23–7.44 (6H, *m*), 7.51–7.68 (1H, *m*), 10.25 (1H, *s*).

2.2.18. Preparation of Tolvaptan Impurity R. To a solution of 2,-methyl 4-nitro benzoic acid (10 g, 0.0552 mol) in toluene (100 mL) and DMF (3 mL) at 0°C thionyl chloride (8.9 mL, 0.0828 mol) was added dropwise. Reaction mixture was heated 60°C for 45 minutes. Solvent and excess thionyl chloride distilled under reduced pressure. In another reaction, to a solution of 1, 2, 3, 4-tetrahydro-benzo[b]azepin-5-one (6.79 g, 0.052) in MDC (100 mL) triethyl amine (27 mL) was added. Isolated acid chloride of 4-nitro benzoic acid was added to the



Scheme 15: Synthetic route for Impurity N.



Scheme 16: Synthetic route for Impurity O.

reaction mixture and stirred for 2 h. RM was washed with DM water (100 mL) and with dilute HCl (100 mL, 10%). Organic layer was separated and dried over sodium sulfate. Solvent was distilled under vacuum to get product as light yellow solid (Scheme 19).

(10 g, % Yield = 55%). HPLC purity = 86.41%, MS *m/z* = 325.11 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.98 (2H, *m*), 2.19 (3H, *s*), 2.77 (2H, *t*), 3.84 (2H, *m*), 6.80–6.93 (1H, *dd, J* = 7.0 Hz, *J* =2.5 Hz), 6.99 (1H, *d, J* = 2.5 Hz), 7.10 (1H, *d, J* = 7.0 Hz), 7.15 (1H, *d, J* = 7.1 Hz), 7.80–7.84 (2H, *d, J* = 7.2 Hz), 7.89 (1H, *d, J* = 7.2 Hz).

2.2.19. Preparation of Tolvaptan Impurity S. To a solution of R (8 g, 0.0246 mol) in ethanol (80 mL), $SnCl_2$ (32 g, 0.142 mol) was added. Reaction mixture was cooled to 0°C and conc. HCl (36 mL) was added dropwise. RM was stirred at room temperature for 16 hours. Reaction mixture was poured into crushed ice. pH of the solution was adjusted to 9 using NaOH solution. Ethyl acetate and stirred for 30 minutes. Organic layer was separated, dried over sodium sulfate, and concentrated under vacuum to get product as light yellow solid (Scheme 20).

(4 g, % Yield = 83%). HPLC purity = 94.13%, MS *m/z* = 295.1 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.98 (2H, *m*), 2.19 (3H, *s*), 2.77 (2H, *t*), 3.84 (2H, *m*), 5.30 (2H, *d*), 6.10–6.13 (1H, *dd, J* = 7.0 Hz, *J* = 2.5 Hz), 6.32 (1H, *d, J* = 2.5 Hz), 6.55 (1H, *d, J* = 7.0 Hz), 6.90 (1H, *d, J* = 7.0 Hz), 7.40–7.44 (2H, *d, J* = 7.1 Hz), 7.59 (1H, *d, J* = 7.2 Hz)

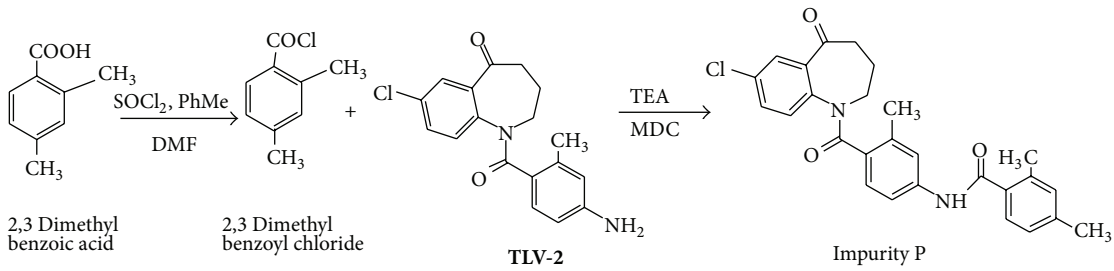
2.2.20. Preparation of Tolvaptan Impurity T. To a solution of S (4 g, 0.0136 mol) in MDC (60 mL) at 0∘C triethyl amine dropwise (5.6 mL, 0.0408 mol). Reaction mixture was stirred at room temperature for 45 minutes. o-Toluoyl chloride (1.95 mL, 0.0149 mol) was added to the reaction mixture and stirred for 1 h. RM was washed with DM water (40 mL) and with dilute HCl (40 mL, 10%). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 21).

(4.5 g, % Yield = 80%). HPLC purity = 92.16%, MS *m/z* = 413.1 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.99 (2H, *m*), 2.30 (3H, *s*), 2.35 (3H, *s*), 2.80 (2H, *m*), 3.71 (2H, *m*), 7.25–7.44 (7H, *m*), 7.51–7.60 (2H, *m*), 10.32 (1H, *s*).

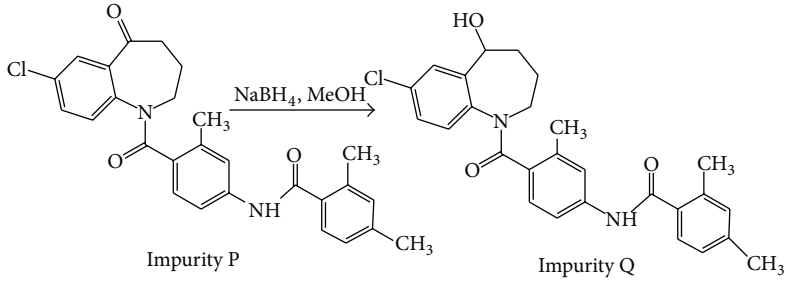
2.2.21. Preparation of Tolvaptan Impurity U. To a solution of T (3.5 g, 0.00849 mol) in methanol (45 mL) at 0°C sodium borohydride (0.157 g, 0.00424 mol) was added portionwise. Solvent of the reaction mixture distilled under vacuum MDC was added to the residue (40 mL) and washed with water (40 mL) and with dil. HCl (40 mL). Organic layer was separated and dried over sodium sulfate. Solvent was distilled under vacuum to get product as light yellow solid (Scheme 22).

(3 g, % Yield = 85%). HPLC purity = 95.18%, MS *m/z* = 415.2 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.98 (2H, *m*), 2.28 (3H, *s*), 2.35 (3H, *s*), 2.80 (2H, *m*), 3.71–3.95 (3H, *m*), 5.41 (1H, *d, J* = 7.0 Hz), 7.28–7.44 (7H, *m*), 7.60–7.65 (2H, *m*), 10.32 (1H, *s*).

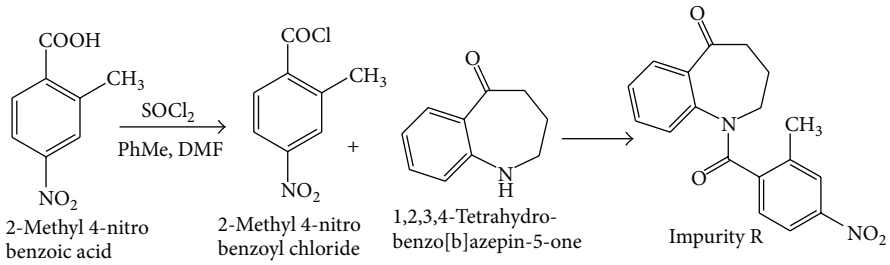
2.2.22. Preparation of Tolvaptan Impurity V. To a solution of benzoic acid (3.0 g, 0.0245 mol) in toluene (30 mL) and DMF (0.1 mL) at 0°C thionyl chloride (2.7 mL, 0.0367 mol) was added dropwise. Reaction mixture was heated 60°C for 45 minutes. Solvent and excess thionyl chloride was distilled under reduced pressure. In another reaction, to a solution



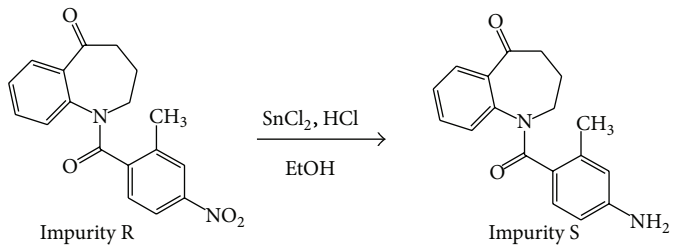
Scheme 17: Synthetic route for Impurity P.



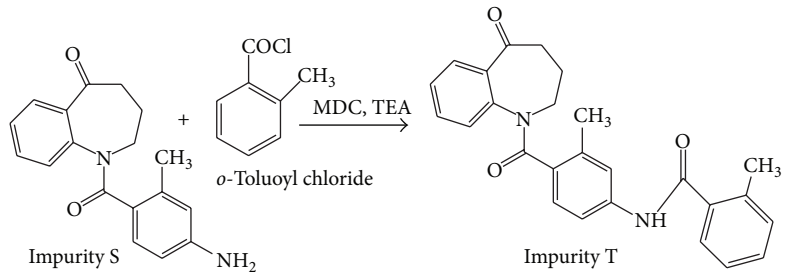
Scheme 18: Synthetic route for Impurity Q.



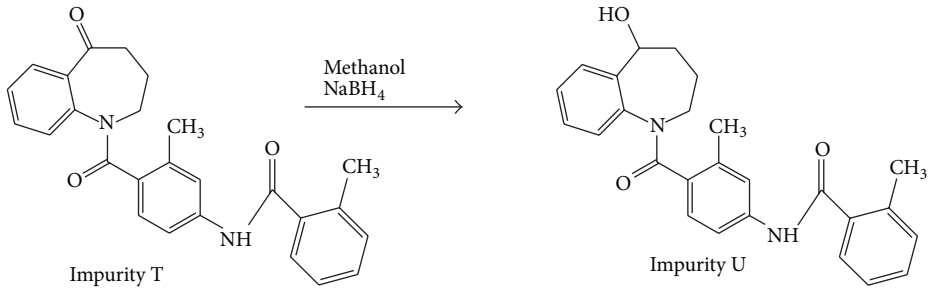
Scheme 19: Synthetic route for Impurity R.



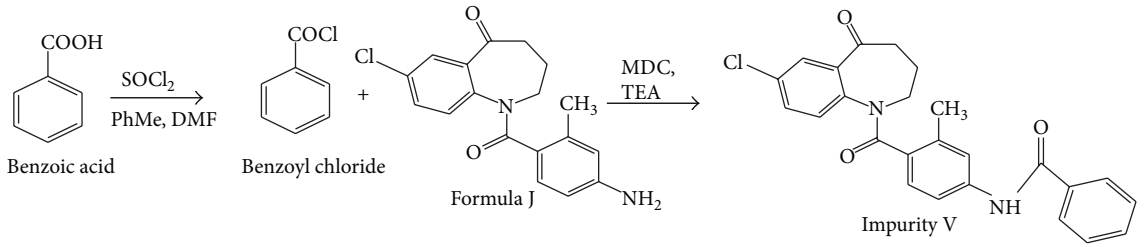
Scheme 20: Synthetic route for Impurity S.



Scheme 21: Synthetic route for Impurity T.



Scheme 22: Synthetic route for Impurity U.



Scheme 23: Synthetic route for Impurity V.

of **TLV-2** (8 g, 0.0243 mol) in MDC (80 mL), triethyl amine (10 mL, 0.0732 mol) was added. Reaction mixture was stirred for 15 minutes. Isolated acid chloride of benzoic acid was added to the reaction mixture and stirred for 45 minutes. RM was washed with DM water (60 mL) and with dilute HCl (60 mL, 10%). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as yellow solid (Scheme 23).

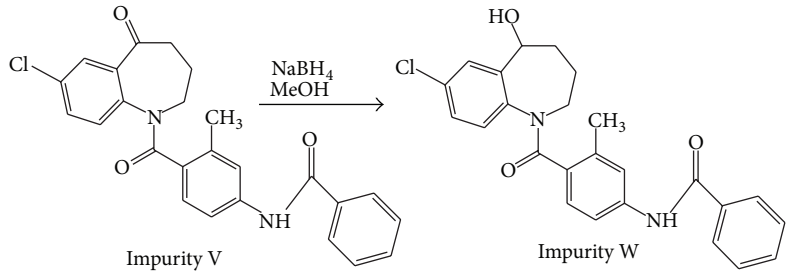
(7.5 g, % Yield = 70%) HPLC purity = 77.7% which is further purified by flash column chromatography to obtain compound of purity 99%. MS m/z = 433.1 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.45–1.68 (2H, *m*), 1.84–1.90 (1H, *m*), 2.10-2.11 (1H, *m*), 2.35 (3H, *s*), 2.60–2.70 (1H, *m*), 4.63–4.67 (1H, *m*), 6.60–6.79 (2H, *m*), 7.02–7.06 (1H, *dd, J* = 7.0 Hz, *J* = 2.8 Hz), 7.24–7.44 (2H, *m*), 7.48–7.61 (3H, *m*), 7.90 (2H, *d*), 10.18 (1H, *s*).

2.2.23. Preparation of Tolvaptan Impurity W. To a solution of V (3.5 g, 0.00810 mol) in methanol (40 mL) at 0°C sodium borohydride (0.5 g, 0.0121 mol) was added portionwise. Reaction mixture was stirred at the same temperature for 0.5 h. Solvent of the reaction mixture was distilled under vacuum. MDC was added to the residue (40 mL) and washed with water (40 mL) and with dil. HCl (40 mL). Organic layer was separated, dried over sodium sulfate, and distilled under vacuum to get product as light yellow solid (Scheme 24).

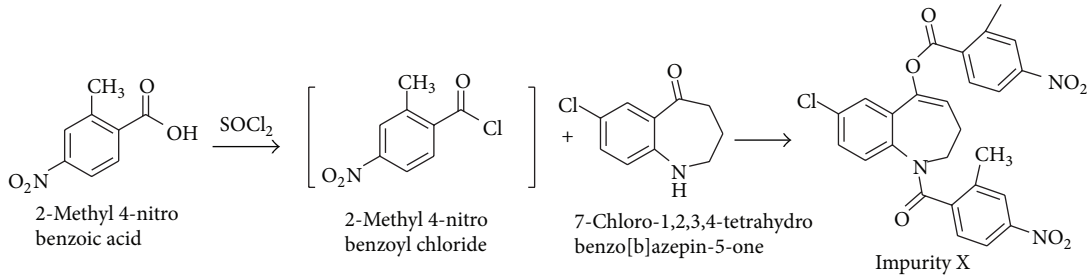
(2.5 g, % Yield = 71%). HPLC purity = 77.27% which is further purified by flash column chromatography. MS m/z = 435.1 (M + H). ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 1.44–1.77 (2H, *m*), 1.94–1.99 (1H, *m*), 2.11–2.14 (1H, *m*), 2.35 (3H, *s*), 2.65–2.73 (1H, *m*), 4.63–4.67 (1H, *m*), 4.80–4.92 (1H, *m*), 5.70 (1H, *d, J* = 7.0 Hz), 6.60–6.79 (2H, *m*), 7.02–7.06 (1H, *dd, J* = 7.1 Hz, *J* = 2.7 Hz), 7.24–7.44 (2H, *m*), 7.48–7.61 (3H, *m*), 7.90 (2H, *d, J* = 7.1 Hz), 10.18 (1H, *s*).

2.2.24. Preparation of Tolvaptan Impurity X. 2-Methyl-4-nitro benzoic acid (20 g, 0.1101 mol) is stirred with Toluene (200 mL) and thionyl chloride (20 g 0.168) with stirring at room temperature for about one hour was added (Scheme 25). During which reaction mixture was heated to reflux for two hours. After confirming the absence starting material solvent was removed by atmospheric distillation. The obtained crude was diluted with dichloromethane, The reaction mass was slowly added to stirred mixture of 7-chloro-1,2,3,4-tetrahydrobenzo[b]azepin-5-one (8.64 g 0.044 mol) in dichloromethane and triethylamine (55 g, 0.5520 mol). Reaction mixture was stirred at room temperature for 6 hours to complete reaction. The resulting reaction mixture was slowly added to a vessel containing 100 mL of 10% sodium chloride under stirring. The organic layer is separated and washed twice with 1 *N* HCl (100 mL) and 5% sodium bicarbonate (100 mL) solution and dried over sodium sulphate, sodium sulfate was removed by filtration, and the filtrate was concentrated under reduced pressure to give 2-methyl-4-nitro-benzoic acid 7-chloro-1-(2-methyl-4-nitro-benzoyl)-2,3-dihydro-1*H*-benzo[b]azeoin-5-yl-ester (Yield 82%) HPLC purity = 99.0%, MS m/z = 522 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 2.68–2.71 (1H, *m*), 2.71 (6H, *s*), 2.91–3.11 (2H, *m*), 4.78–4.82 (1H, *m*), 6.94 (1H, *d, J* = 7.2 Hz), 7.15–7.18 (1H, *dd, J* = 7.3 Hz, *J* = 2.6 Hz), 7.43–7.48 (1H, *m*), 7.79 (1H, *m*), 8.09 (1H, *s*), 8.23–8.27 (1H, *dd, J* = 7.3 Hz, *J* = 2.6 Hz), 8.32 (1H, *d, J* = 7.4 Hz), 8.41 (1H, *d, J* = 7.2 Hz).

2.2.25. Preparation of Tolvaptan Impurity Y. To a stirred mixture of methanol (240 mL) and concentrated HCl (84 mL), 2-methyl-4-nitro-benzoic acid 7-chloro-1-(2-methyl-4-nitro-benzoyl)-2,3-dihydro-1*H*-benzo[b]azeoin-5-yl-ester (12 g 0.023 mol) was added and $SnCl_2 \cdot 2H_2O$ (42 g



Scheme 24: Synthetic route for Impurity W.



Scheme 25: Synthetic route for Impurity X.

0.186 mol) with stirring at room temperature was added (Scheme 26). The reaction mass was maintained at reflux temperature for 2 hours. After confirming the disappearance of starting material, the crude obtained was diluted with dichloromethane and added water. The biphase obtained adjusted the pH of reaction mass 12–14 using 40% NaOH solution. Layer was washed with water (2 × 100 mL) and sodium bicarbonate (2 × 100 mL) and dried over sodium sulfate, after filtering off sodium sulfate; the filtrate was concentrated under reduced pressure to give the crude product. The resultant crude was with ethyl acetate to give 4-amino-2-methyl-benzoic acid 1-(4-amino-2-methyl-benzoyl)-7-chloro-2, 3-dihydro-1*H*-benzo[b]azepin-5-yl-ester. The crude product purified in column chromatography using 60–120 mesh silica gel and product was eluted in 30% ethyl acetate in Hexane.

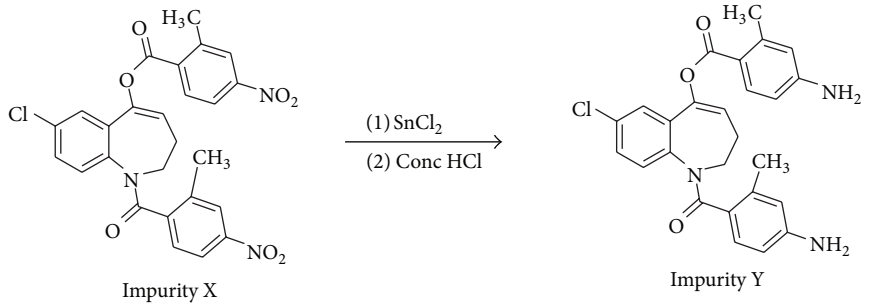
(Yield-56%) (HPLC-92.0%), MS m/z = 462 (M + H), ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 2.25 (3H, *s*), 2.45 (3H, *s*), 2.58-2.59 (1H, *s*), 2.72–2.79 (2H, *s*), 4.79 (1H, *m*), 6.03–6.08 (4H, *m*), 6.29 (1H, *d, J* = 7.0 Hz), 6.47–6.54 (2H, *m*), 6.70 (1H, *d, J* = 7.2 Hz), 6.77 (1H, *d, J* = 7.2 Hz), 7.08–7.12 (1H, *dd, J* = 7.2 Hz, *J* = 2.6 Hz), 7.29 (1H, *d, J* = 7.1 Hz), 7.95 (1H, *d, J* = 7.1 Hz).

2.2.26. Preparation of Tolvaptan Impurity Z. 4-Amino-2-methyl-benzoic acid 1-(4-amino-2-methyl-benzoyl)-7-chloro-2,3-dihydro-1*H*-benzo[b]azepin-5-yl-ester (5 g 0.0108 mol) was dissolved in dichloromethane (50 mL) and triethylamine (7.5 mL 0.054 mol) and O-toluoyl chloride (4.17 g 0.027 mol) was added with stirring under ice cooling over a period of 30 minutes (Scheme 27). After confirming the disappearance of starting material, dichloromethane layer was washed with water (2 × 50 mL) and 5% sodium bicarbonate (2 × 50 mL) and dried over sodium sulfate; after filtering off sodium sulfate, the filtrate was concentrated under reduced pressure to give the crude product. The resultant crude was recrystallized in isopropyl ether to give 2-Methyl-4-(2-methyl-benzoylamino)-benzoic acid 7-chloro-1-[2-methyl-4-(2-methyl-benzoylamino)benzoyl]-2, 3-dihydro-1*H*-benzo[b]azepin-5-yl-ester.

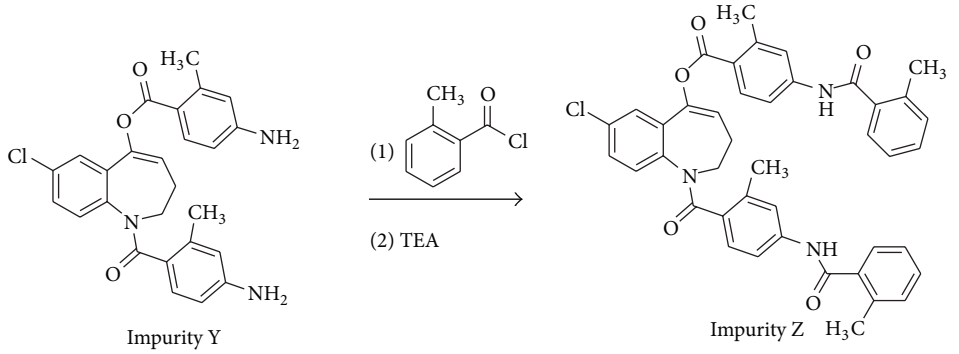
(Yield-65%) (HPLC-98.0%) MS *m/z* = 698 (M + H) ^{1}H NMR (300 MHz, DMSO-d_6, δ/ppm) 2.35 (3H, *s*), 2.40 (6H, *s*), 2.59 (3H, *s*), 2.59–2.67 (1H, *m*), 2.87–3.01 (2H, *m*), 4.82–4.85 (1H, *m*), 6.26 (1H, *m*), 6.83 (1H, *d*), 7.12–7.18 (1H, *m*), 7.27–7.51 (12H, *m*), 7.81–7.84 (2H, *m*), 8.25 (1H, *d, J* = 7.1 Hz), 10.26 (1H, *s*), 10.66 (1H, *s*).

3. Results and Discussion

This impurity (Impurity A) could be formed when unreacted 7-Chloro-1,2,3,4-tetrahydro-benzo[b]azepin-5-one from the first stage reacts with o-toluoyl chloride which is added in the third stage. Impurity B is possible when Impurity A is carried over to the fourth stage and gets reduced. Impurity C is possible when unreacted 2-methyl 4-nitro benzoic acid is reduced and then condensed with o-toluoyl chloride in the third stage. Complete consumption of 2-methyl 4-nitro benzoyl chloride would limit the formation of this impurity. Impurity D is possible if unreacted **TLV-2** is carried forward to the fourth step. Impurity E is possible if unreacted o-toluoyl chloride reacts with the Tolvaptan (**TLV-4**). Impurity F is possible when 4-nitro benzoic acid is present as an impurity in starting material 2-methyl 4-nitro benzoic acid. Impurity G is possible if Impurity F is carried forward to the second stage. Both Impurities F and G could be avoided by using 2-methyl 4-nitro benzoic acid which is free of 4-nitro



Scheme 26: Synthetic route for Impurity Y.



Scheme 27: Synthetic route for Impurity Z.

benzoic acid impurity. Impurity H is possible when impurity G is carried forward to the next step. Impurity I is possible when impurity H is carried forward to the next step (Stage 4). Impurity J is possible when 4-methyl benzoic acid is present as an impurity in 2-methyl 4-nitro benzoic acid. Impurity K is possible when impurity J is carried forward to the next step (Stage 4). Impurity L is possible when 3-methyl benzoic acid is present as an impurity in 2-methyl 4-nitro benzoic acid. Impurity M is possible when Impurity L is carried forward to next step (Stage 4). Impurity N is possible when 3-4, dimethyl benzoic acid is present as an impurity in 2-methyl 4-nitro benzoic acid. Impurity O is possible when impurity N is carried forward to the next step (Stage 4). Impurity P is possible when 2,3-dimethyl benzoic acid is present as an impurity in 2-methyl 4-nitro benzoic acid. Impurity Q is possible when Impurity P is carried forward to the next step (Stage 4). Impurity S is possible when Impurity R is carried forward to the second step.

Impurity T is possible when Impurity *S* is carried forward to the third step. Impurity U is possible when Impurity T is carried forward to the fouth step. Impurity V is possible when benzoic acid is present as an impurity in o-toluoyl chloride and could be formed in the third stage. Impurity W is possible when Impurity V is carried forward to the fourth step. This impurity is observed at 0.97 RRT in the LC-MS of **TLV-4**. Impurity X is formed when 7-chloro-1,2,3,4-tetrahydrobenzo[b]azepin-5-one is reacted with 2-methyl 40 nitro benzoyl chloride in presence of a base such as triethyl amine. In order to avoid formation of this impurity the reaction is carried out in absence of base. This impurity is observed at 1.36 RRT in the chromatogram of the first stage. Impurity Y is possible when Impurity X is carried forward to the second step. Impurity Z is possible when Impurity Y is carried forward to the fourth step. This impurity is observed at 1.44 RRT in the chromatogram of the third stage.

4. Conclusion

In conclusion different observed as well as potential impurities formed during the process for Tolvaptan have been described with their synthetic procedure and characterization data. International Conference on Harmonization (ICH) has formulated guidelines regarding the control of impurities and hence impurity profiling is a critical issue to the pharmaceutical industry. Keeping in view the regulatory importance of Tolvaptan, synthesis and characterization of these impurities could be highly beneficial. This paper outlines the description of different impurities related to Tolvaptan and their methods of synthesis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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