Novel Synthesis, Identification and Characterization of Process Related Impurities of Ractopamine Hydrochloride

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Abstract:

A synthesis process has been developed to produce high purity Ractopamine Hydrochloride & its impurities for the verification as per the regulatory requirement for Active Pharmaceutical Ingredients (API). For stress study analysis to reveal the plausible impurities and its cause of generation. In this article we have developed a novel method for the synthesis of Deoxy ractopamine impurity and *o*-methyl ractopamine impurity of Ractopamine Hydrochloride. Both the impurities are process related impurities and formed during API synthesis. Further these impurities were isolated and characterized by LC-MS, and NMR.

Keywords:

Ractopamine HCl, Deoxy ractopamine and *o*-methyl ractopamine impurity.

Introduction:

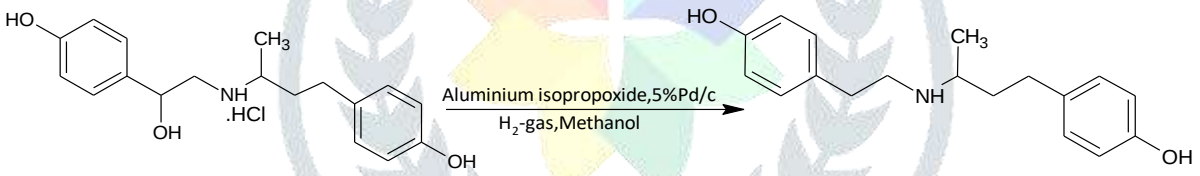
Ractopamine HCl is a beta-adrenergic leanness-enhancing agent recently approved for use in swine. The drug was first reported by Elinco animal division of Eli Lilly and company [1].Several synthesis method were reported for Ractopamine [2-4].Most common synthetic method involves reductive amination of raspberry ketone as the starting key raw material with 4-(2-amino-1- hydroxyethyl) phenol. Even though the drug is banned in other countries due to residual drug concentrations detected in swine tissue by, the drug has great market in US under the brand name of Paylean for swine, Optaflexx for cattle, and Topmax[5] for Turkeys. Pharmacologically, it is a phenol-based TAAR1 agonist and β adrenoreceptor agonist that stimulates β_1 and β_2 adrenergic receptors. It is most commonly administered to animals for meat production as Ractopamine hydrochloride. To meet the regulatory guidelines, this drug is produced by the route described in literature was freezed for its complete analytical evaluation and impurity

profiling. Since impurity profiling of a drug substance is very critical task for its safety assessment and manufacturing, it is mandatory to identify and characterized the impurities in pharmaceutical product [6-8].

Materials and Method:

All the key raw materials used for synthesis were obtained from,SigmaAldrich and was used without further purification. All other solvents and reagents used were of analytical or HPLC grade. NMR was recorded on Bruker Avance 400 MHz NMR Spectrometer, chemical shifts are reported in ppm downfield from internal standard tetramethylsilane (TMS). IR was analysed on Thermo Scientific Nicolet iS50 FT-IR Spectrometer.Mass analysis was performed on Thermo Scientific LC-MS Orbitrapbased systems and Shimadzu LC-MS-2010 EV. Thin-layer chromatography (TLC) Merck TLC Silica Gel 60 F254, (0.25 mm) detection: UV light at 254 nm. Plates were visualized by UV light and iodine vapour. HPLC analysis was performed in Waters Pump–alliance (2695), auto sampler- alliance (2695); detector-UV (2489); with Empower software or equivalent Inertsil ODS-3, 150 x 4.6 mm, 5µm, Flow rate -0.8 ml/minute, Column oven Temperature 40°C, Detector UV 257 nm, Injection volume 20.0 ml, Run time 40 minutes, Needle Wash Water: Acetonitrile (20:80 v/v), Diluent Buffer: Acetonitrile(50:50 v/v). Flash chromatography: Teledyne Isco Combiflash Rf+ Lumen (230-400 mesh), detector: ELSD.

A) Synthesis of Deoxy Ractopamine Impurity:



Ractopamine Hydrochloride Deoxy Ractopamine

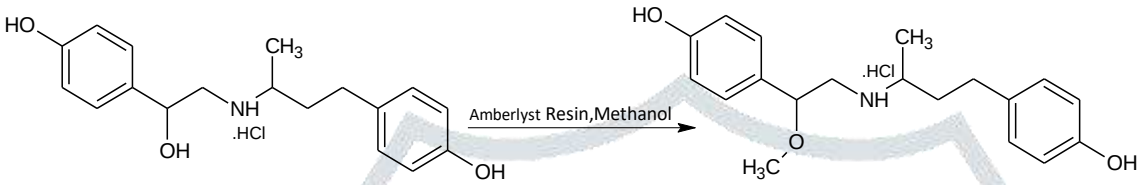
Scheme: 1

Experimental procedure: In a 2 L capacity S.S 316 L autoclave Ractopamine hydrochloride (50.0g), was charged followed by methanol (800 ml), 5%Pd/c (5.0g) and aluminium isopropoxide (10.0 g). The reaction was flushed with nitrogen to remove the dissolved oxygen. The reaction mass was heated to 40-45^{0}C. At 40-45^{0}C the vessel was pressurised with hydrogen gas at (4.0kg/cm^2). The reaction was maintained for 8.0 hr. The completion of reaction was monitored by TLC. After completion of reaction, the catalyst was filtered on celite bed the mother liquor was distilled under reduced pressure to obtain crude oil. The crude oil was charged in flask containg a mixture of ethyl acetate (700 ml) and 10% ammonium hydroxide solution (300ml) and was stirred for 30 minutes. After separation of ethyl acetate the organic layer was distilled under reduced pressure to obtain off white solid powder and crystallised in alcoholic solvent.

Yield (36.5g / 86.4%)

^{1}H NMR: δ 1.17 (3H, d, *J* = 6.7 Hz), 1.55-1.67 (2H, 1.61 (td, *J* = 7.2, 6.0 Hz), 1.61 (td, *J* = 7.2, 6.0 Hz)), 2.51-2.78 (7H, 2.66 (qt, *J* = 6.7, 6.0 Hz), 2.58 (t, *J* = 7.3 Hz), 2.55 (t, *J* = 7.2 Hz), 2.55 (t, *J* = 7.2 Hz), 2.58 (t, *J* = 7.3 Hz), 2.74 (t, *J* = 7.3 Hz), 2.74 (t, *J* = 7.3 Hz)), 6.67-6.74 (4H, 6.70 (ddd, *J* = 8.2, 2.5, 0.5 Hz), 6.70 (ddd, *J* = 8.2, 2.6, 0.5 Hz)), 6.99-7.06 (4H, 7.03 (ddd, *J* = 8.2, 1.0, 0.5 Hz), 7.02 (ddd, *J* = 8.2, 1.0, 0.5 Hz)).

B) Synthesis of O-Methyl Ractopamine Impurity:



Ractopamine Hydrochloride O-Methyl Ractopamine

Scheme: 2

Experimental procedure: In a 2 L 4 neck RBF fitted with overhead stirrer and reflux condenser, ractopamine hydrochloride was charged followed by methanol (800ml) and Amberlyst acidic resin (5.0 g). The reaction mass was heated at 55-60^{0}C.After 8.0 hr reaction was filtered through celite bed the solvent was distilled under reduced pressure to get crude oil. Charged ethyl acetate (300 ml) in Crude oil and Distilled out under reduced pressure. Charged (300ml) ethyl acetate in crude material and filter to get desired product.

Yield: 43.70 g/ 84%.

^{1}H NMR: δ 1.18 (3H, d, *J* = 6.7 Hz), 1.56-1.67 (2H, 1.61 (td, *J* = 7.2, 6.0 Hz), 1.61 (td, *J* = 7.2, 6.0 Hz)), 2.51-2.59 (2H, 2.55 (t, *J* = 7.2 Hz), 2.55 (t, *J* = 7.2 Hz)), 2.73 (1H, qt, *J* = 6.7, 6.0 Hz), 2.93-2.97 (2H, 2.95 (d, *J* = 5.8 Hz), 2.95 (d, *J* = 5.8 Hz)), 3.25 (3H, s), 4.57 (1H, t, *J* = 5.8 Hz), 6.66-6.74 (4H, 6.69 (ddd, *J* = 8.2, 1.4, 0.6 Hz), 6.70 (ddd, *J* = 8.2, 2.6, 0.5 Hz)), 7.02 (2H, ddd, *J* = 8.2, 1.0, 0.5 Hz), 7.25 (2H, ddd, *J* = 8.2, 1.2, 0.6 Hz).

RESULTS AND DISCUSSION:

The impurity detection was analysed on HPLC of Ractopamine .HCl samples were subjected to HPLC analysis using USP HPLC method. Standard Deoxy ractopamine impurity was observed at 1.10 RRT. As per regulatory guidelines requirement API must be subjected to stress test for detailed characterization. No major degradant were seen in oxidation, aqueous and thermal stress but several impurity peaks were formed in acid and Base hydrolysis. Deoxy ractopamine impurity was formed during hydrogenation reaction 0.10% to 0.25% maximum. This impurity was controlled during hydrogenation reaction.

O-methyl ractopamine impurity was process impurity observed at 1.20 RRT. Formation of this impurity was observed in final stage approx. 0.05% to 0.20% range.

Deoxy ractopamine analytical data shows that the elemental compositions are consistent with predicted structure (Shown in Scheme: 1). The IR and PMR spectral data also support the predicted structure. The structures assigned to Deoxy Ractopamine were supported by the analytical data. Infra-Red and PMR spectra showing an absorption bands at 3020-3080cm^{-1} (C-H of Ar.), 2950, 2878, 1370cm-^{1} (-CH3, CH2), 3500cm^{-1} –NH. Mass spectra shows ES (M+H):286.28.

O-Methyl ractopamine impurity analytical data shows that the elemental compositions are consistent with predicted structure (Shown in Scheme: 2). The Infra-Red and PMR spectral data also support the predicted structure. The structures assigned to O-methyl ractopamine impurity supported by the analytical data. Infra-Red and PMR spectra showing an absorption bands at 3020-3080cm^{-1}(C-H of Ar.), 2950, 2878, 1370cm-^{1} (-CH3, CH2), 3500cm^{-1} –NH, and R-O-R 1100 cm^{-1}. Mass spectra shows ES (M+H):316.35.

CONCLUSION:

Deoxy Ractopamine was formed during ketoractopamine to ractopamine preparation in hydrogenation reaction. In this reaction formation of deoxy ractopamine impurity maximum 0.25% and this impurity limit was 0.50% as per ICH guidelines. Deoxy ractopamine impurity was synthesised by novel method using Aluminium ispoproxide reagent. Aluminium isopropoxide was easy to handle and cheaper reagent. Secondary alcohol was easily dehydration by this reagent.

In acidic media O-Methyl ractopamine impurity was formed during work up of final stage. In this stage methanol was used. Formation of impurity maximum. 0.20% and limit of this impurity as per ICH guideline is 1.0%. Acidic amberlyst resin was used for synthesis of O-methyl ractopamine Impurity.

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