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IDENTIFICATION, CHARACTERIZATION AND SYNTHESIS OF IGURATIMOD PROCESS AND DEGRADATION RELATED IMPURITIES

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ABSTRACT

A synthesis process developed to produce safe and high purity Iguratimod API. As per regulatory requirement the obtained API subjected to stress test for profiling the plausible impurities and its route of formation. During these studies total of four impurities related to process, forced degradation and OVI were observed. Three of these impurities were detected by HPLC and one by GC. Further these impurities were isolated and subjected to LC-MS, GC-MS and NMR studies. For absolute confirmation these impurities were synthesized and profiled.

KEYWORDS: Iguratimod, methyl impurity, 5-phenoxybenzoic acid impurity, amine impurity, acetaimidine.

1.0 INTRODUCTION

Iguratimod (IGRD), chemically known as N-[(formylamino)-4-oxo-6-phenoxy-4Hchromen-7-yl] methanesulfoanamide, is an anti inflammatory agent used for the treatment of rheumatoid arthritis. Iguratimod was first Reported in product patent US4954518.[1] Its Therapeutic category is Anti-arthritic and novel immunomodulator.[2] Iguratimod is a nuclear factor NF-κB activation inhibitor used in the treatment of rheumatoid arthritis. It also suppressed inflammatory cytokine production in cultured human synovial cells induced by tumor necrosis factor (TNF)-α by inhibiting the activity of nuclear factor-κB.

Several synthesis processes are reported for IGRD.[3-6] After safety and reaction hazard evaluation it was observed that most of the processes were using non-feasible reaction conditions such as use of Pyridine as solvent, bromination, azidation and strong base like potassium tert. Butoxide at elevated temp. It is also found that the earlier process were of low yield and mediocre quality. Present research work involves development of a new synthesis route which is safe in operation and commercially viable in terms of yield and above par in quality.[7] To meet the regulatory guidelines, IGRD produced by this route was subjected to complete analytical evaluation and impurity profiling. Impurity profile of a drug substance is critical for its safety assessment and manufacturing process. It is mandatory to identify and characterize the impurities in the pharmaceutical product, if present above the accepted limits of 0.1 %.[8]

Hence authors have extensively examined the impurities, by-products and degradants using spectroscopic and spectrometric tools such as gas/liquid chromatography, IR, Mass and NMR.

2.0 MATERIALS AND METHODS

All the raw materials used for synthesis were obtained from Aldrich, Merck and Sigma used without further purification. All other chemicals were of analytical or HPLC grade. Proton magnetic resonance ^{1}H NMR: Bruker Avance 400 MHz NMR Spectrometer, chemical shifts are reported in ppm downfield from internal standard tetramethylsilane (TMS). IR: Thermo Scientific Nicolet iS50 FT-IR Spectrometer and MS: Thermo Scientific LC-MS Orbitrap-based 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 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 ㎖, 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, GC: Perkin Elmer Clarus 500/580, Head Space sampler: Turbo Matrix HS-40, Column: DB-624 Capillry column (30 m x 0.53mm x3.0µ m), Detector: FID, Injector temp. 140^{0}C, Detector temp. 240, Carrier gas Nitrogen, Split ratio 5:1, Carrier gas flow 2.0 ml/min, Range 1, Attenuation -6, Run time 37.5 min.

3. RESULTS AND DISCUSSION

3.1 *Detection of impurity by HPLC and GC*

IGRD samples were subjected to HPLC analysis using HPLC method mentioned in section 2.0. IGRD peak appeared at 17.4 min along with several other impurities. However an impurity at RT 20.6 min was above the threshold limit of 0.1%. This impurity peak was designated as IMP-I. Hence a complete characterization of this impurity peak was required.

As per regulatory guidelines requirement API must be subjected to stress test for detailed characterization.[9-10] No major degradant were seen in oxidation, aqueous and thermal stress but several impurity peaks were formed in Acid and Base hydrolysis. When compared with reported data most of them were matching with either precursor or intermediated or reported known impurities. However peaks at 7.7 min and 14.6 were not matching with any of the earlier IGRD related moiety. These impurities were marked as IMP-II & IMP-III resp.

Another critical tool, gas chromatographic was incorporated to determine the volatile and solvent related impurities in API (OVI). When IGRD subjected for OVI using GC method mentioned in section 2.0, a major unknown peak appeared at 13.2 min apart from known solvents. This impurity was labeled as IMP-IV.

3.2 Structural elucidation of IMP-I, II & III by LC-MS

IGRD sample containing impurity IMP-I above 0.1% was subjected to LC/ESI/MS (positive mode/negative mode) and MS/MS analysis. IGRD mass data gave m/z peak 373 in $[M-H]^-$. While MS/MS study of IMP-I showed ion peaks at m/z 389 in $[M+H]^+$. MS/MS analysis did not give any further fragment. The difference is of 14 Da between IGRD and IMP-I. In ^1H nmr singlet peak appeared at 3.3 ppm which correspond to 3H and carbon peak at 39.6-40.6 Combining together this indicated presence of two methyl group as compared to one in IGRD. Considering chemical shift in NMR and theoretical possibility it is concluded that the methyl group is attached to nitrogen atom of sulfonamido group of IGRD. Such impurity is reported.[11] but its verification by synthesis is not done. In present research work authors have synthesized it by novel route.

FD samples showed IMP-II & III were analyzed by LC-MS. IMP-II gave ion peak at m/z 322 & MS/MS gave fragment ion peak at m/z 278 in $[M-H]^-$, loss of 44 Da. Odd molecular mass 323 indicated that the molecule contains odd number of nitrogen atom in structure. IGRD has one cromone ring and gives allylic singlet proton signal between 8 to 9 ppm. This signal was

missing in IMP-II. In combination with mass and NMR data it is concluded that the formamide side chain is severed and cromone ring is opened. Opening of cromone ring created two new functional group carboxylic acid and hydroxyl.[12-14] This carboxylic side chain was seen in MS/MS fragment loss of 44 Da. Taking together structure of IMP-II was revealed as 2-hydroxy-4-(methylsulfonamido)-5-phenoxybenzoic acid.

While IMP-III gave ion peak at m/z 345 and MS/MS gave fragment ion peak at m/z 267 in $[M-H]^-$, loss of 78 Da. Even mass of molecule indicated presence of either absence or even number of nitrogen atoms. It is also noted that this impurity is 28 Da less than parent molecule confirming detachment of aldehyde side chain but even mass confirmed retention of amine group from formamide side chain. Loss of 78 Da amount to cleaving of methyl sulfonyl moiety. ^1H and ^{13}C NMR spectra confirmed the absence of aldehyde carbon beyond 180 ppm and proton signal beyond 8 ppm in ^1H spectra. Combining together mass and NMR data derived IMP-III structure as N-(3-amino-4-oxo-6-phenoxy-4H-chromen-7-yl) methanesulfonamide.

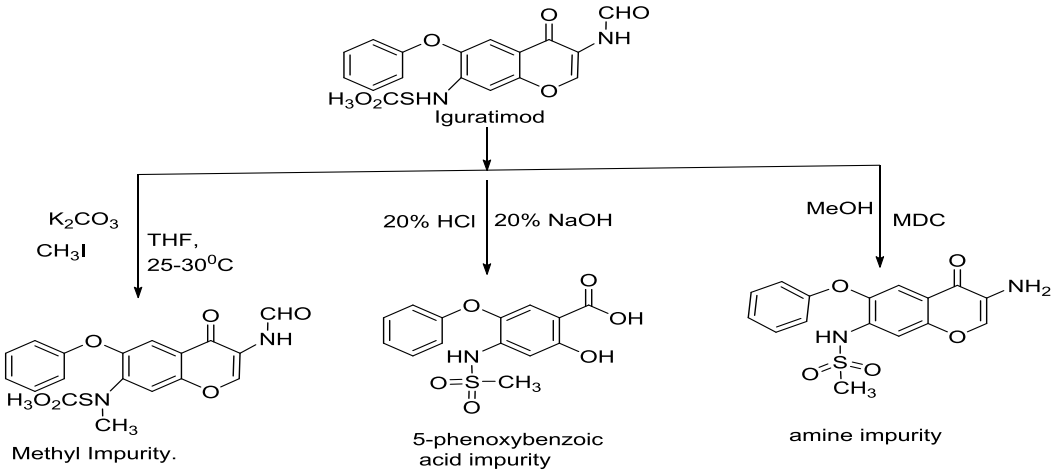
Impurity I, II & III were confirmed by their synthesis as given in Scheme I.

3.3 Structural elucidation of IMP- IV GC-MS

GC-MS analysis of OVI impurity IMP-IV gave molecular ion peak at m/z 86. This was no where close to parent molecule and has even mass. 1H analysis showed only two sharp singlets integrating to 3 and 6 protons. Investigating further it is found that the N, N-dimethyl formamide dimethyl acetal / N, N-dimethyl amine HCl and acetonitrile were used as reagent and solvent. Condensation reactions of these two reagent and solvent are known during acid base purification of IGRD.[15-17] Based on reaction history, mass and NMR data IMP-IV was concluded as N, N-dimethyl ethanimidamide.

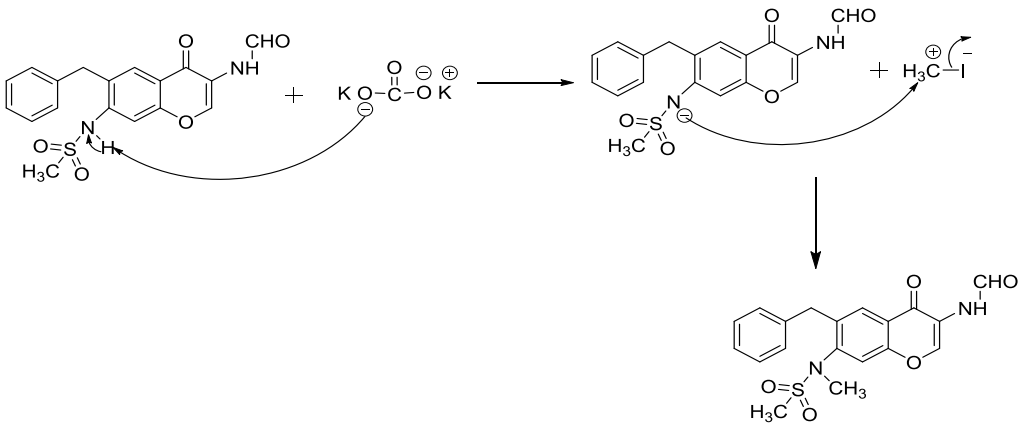
All the impurities proposed in present research work were confirmed by their synthesis as given in Scheme V and re-injecting to respected chromatographic system to verify.

3.4 Detailed reaction sequence with Formation and synthesis of IMP-I, II, III & IV

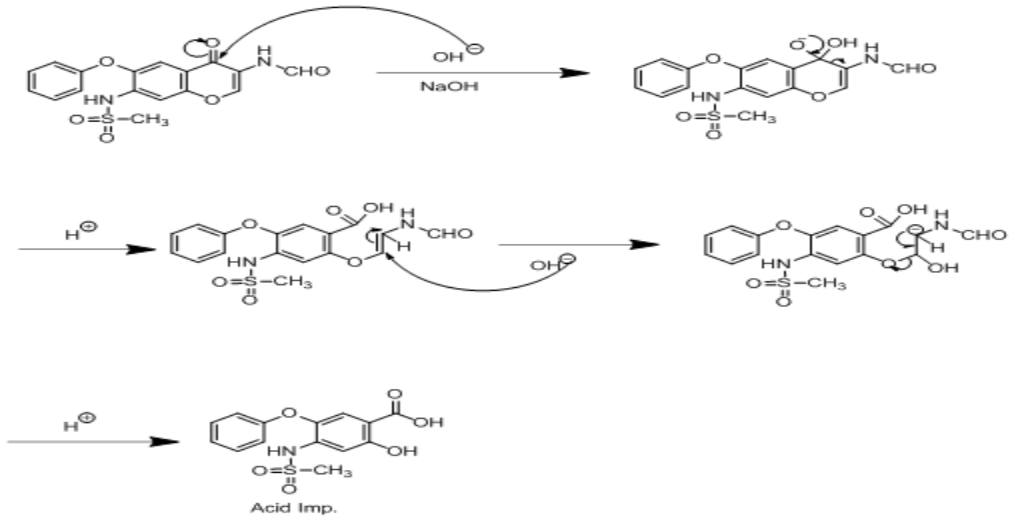


IMP-I **IMP-II** **IMP-III**

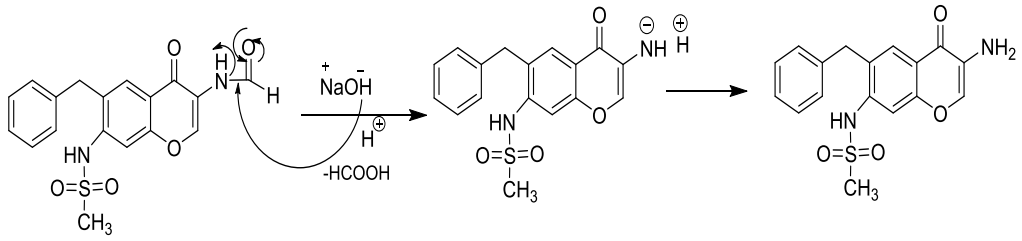
Scheme I: Synthesis of Impurities IMP-I, IMP-II, IMP-III.



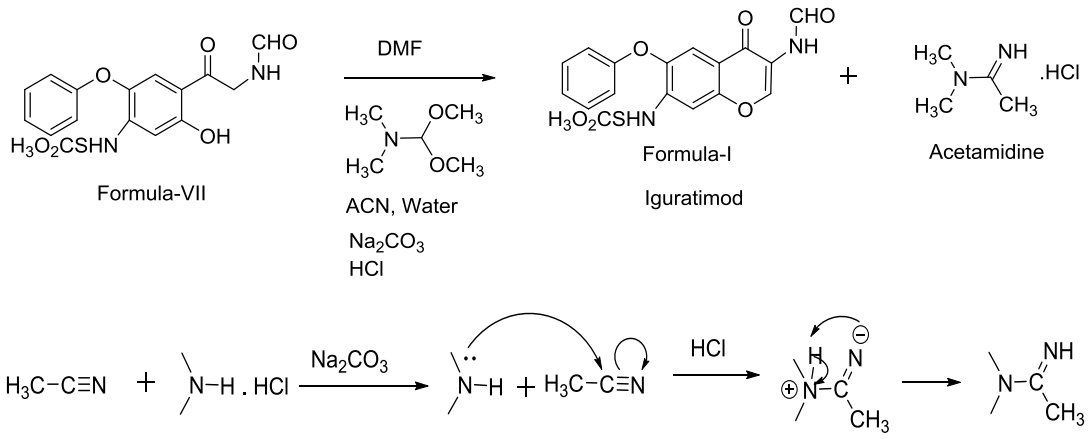
Scheme II: Plausible reaction Mechanism of the Desired methyl impurity (IMP-I).



Scheme III: Plausible reaction Mechanism of the Desired 5-phenoxybenzoic acid impurity (IMP-II).



Scheme IV Plausible reaction Mechanism of the Desired amine impurity (IMP-III).



Scheme V Plausible reaction Mechanism of the Desired Acetamidine impurity (IMP-IV)

3.5 *Formation and synthesis of IMP-I, II, III & IV*

Synthesis of IMP-I

When IGRD pure (10.0g), Methyl iodide (4.176g) and Potassium carbonate (11.0g) were charged in 50 ml THF at room temperature. The reaction mass was stirred for 18-20 hrs. Precipitated solid (white) was filtered and washed with water (50 ml) and dried at 100 °C for 8 hrs. Obtained Methyl impurity (IMP-I). Mechanism of formation is discussed in **scheme I** and **II.**

Anal. Calc. for $C_{18}H_{16}N_2O_6S$, Mol. Wt.: 388.3944, C: 55.66; H: 4.15; N: 7.21; O: 24.72; S: 8.26, **^{1}H NMR:** 400 MHz, DMSO: δ ppm:3.17 (s, 3H, -CH_3), 3.30 (s, 3H, -CH_3), 7.20-7.22 (D, 2H, -Ar), 7.27-7.31 (m, 1H, -Ar), 7.32 (s, 1H, -Ar),7.48-7.52 (m, 2H, -Ar), 7.95 (s, 1H, -Ar),8.33 (D, 2-1H, -CHO), 9.37 (s, 1H, -Ar), 9.95 (s, 1H, -NH). **^{13}C NMR:** 400 MHz, DMSO: δ ppm: **^{13}C NMR:** 400 MHz, DMSO: δ ppm: 38.1-39.3, 39.6-40.6, 111.7, 120.3, 121.3, 122.2, 123.9, 125.5, 131.0, 138.1, 146.7, 151.0, 152.6, 155.8, 161.0, 170.4, **Mass:** m/z 389 in $[M+H]^+$.

Synthesis of IMP-II

Charge 2.0gm Iguratimod, 20 ml 20% NaOH solution at 25°C. Reaction mass becomes light brown color as time passes within 1 hr, maintained at 25°C for 6 hrs. After 6 hrs TLC checked, shows completion of reaction. (Mobile phase MDC: MeOH 9.5:0.5 ml) pH of the reaction mass was adjusted to 2 by using Conc. HCl and stirred at 25°C for 1 hr. Reaction mass was filtered and solid was washed with water. Dry the solid on Rota flask under vacuum at 50-55°C for 6 hrs. Dry Wt. 2.0 g crude HPLC purity 96.78% was purified by Flash Chromatography condition: A1: Hexane & B1: Ethyl acetate, Wavelength: 1) 254 nm & 2) 280 nm (By default), Flow rate: 30 ml/min., Column: 80 gm Silica flash column 400 mg-2.0 gm sample, gave pure IMP-II of Iguratimod 1.6 gm (Yield 93 %). HPLC purity 99.11% Mechanism of formation is discussed in **scheme III.**

Anal. Calc. for $C_{14}H_{13}NO_6S$, Mol. Wt.: 323.3211, C: 52.01; H: 4.05; N: 4.33; O: 29.69; S: 9.92, IR ν(cm1):, **^{1}H NMR:** 400 MHz, DMSO: δ ppm:3.10(s, 3H, -CH_3), 6.97-6.99(m, 2H, -Ar), 7.08(s, 1H, -Ar), 7.10-7.14(m, 1H, -Ar), 7.25(s, 1H, -Ar), 7.36-7.40(m, 2H, -Ar), 9.77(s, 1H, --NH), 11.54(s, 1H, -COOH) , **^{13}C NMR:** 400 MHz, DMSO: δ ppm:39.3-41.0, 108-108.8, 118.1, 120.9, 123.6, 130.3, 137.2,139.0,157.7, 158.6, 171.2, **IR ν(cm1):** 690.64m, 704.85m 760.84m, 794.88m, 852.74m, 926.40s, 972.13w,1071.67s, 1127.93w, 1187.73w, 1216.04w, 1271.04s, 1321.02w, 1432.78s, 1490.01m, 1503.43s, 1589.80s, 1627.11s, 1668.1s, 3247.31s, **Mass:** m/z 322 in $[M-H]^-$.

Synthesis of IMP-III

Charge IGRD 2.0 gm, add 50ml 5N Conc. HCl at 30°C and heated the Reaction mass to 60°C for 7 hrs, shows HPLC analysis complies, Cool the reaction mass slowly to 30-35°C, Filter the reaction mass & wash the solid with 25 ml water. Unload the solid & dried over rota evaporator under vacuum at 50°C for 2 hrs. Dry Wt. 2.5 gm crude having HPLC purity 95.07%. Add 50ml Methanol and heat to 60°C, Cool the reaction mass slowly to 40-45°C, in rota evaporator and stripped out under vacuum 2 times with methanol and degassed completely. Add 25 ml methanol and heat to 60-65°C. Distill the half of the volume of methanol. Cool the reaction mass slowly to 30-35°C for 30 min. Filter & washed the solid with 25 ml methanol. Dried over Rota evaporator under vacuum over at 60-65°C for 6 hrs gave IMP-III with Yield 1.4 g (75.6%). Mechanism of formation is discussed in **scheme IV.** **Anal. Calc.** for $C_{16}H_{14}N_2O_5S$, Mol. Wt.: 346.3578, C: 55.48; H: 4.07; N: 8.09; O: 23.10; S: 9.26, **^{1}H NMR:** 400 MHz, DMSO: δ ppm: 3.25(s, 3H, -CH_3), 5.38(s, 3H, -NH_2), 7.16-7.18(m,

2H, -Ar), 7.25-7.29(m, 2H, -Ar), 7.46-7.50(m, 2H, -Ar), 7.74(s, 1H, -Ar), 8.64(s, 1H, -Ar), 10.21(s, 1H, -NH_2), **^{13}C NMR:** 400 MHz, DMSO: δ ppm: 39.3-41.1, 109.5, 111.0, 118.6, 120.2, 121.0, 125.3, 130.8, 136.0, 146.7, 149.0, 152.4, 155.9, 171.3, **Mass:** m/z 347 in $[M+H]^+$

Synthesis of IMP-IV

Charged Dimethyl amine HCl 5.0 g in 50 ml Methanol and adjusted pH8-9 by using sodium methoxide at 0-5^{0}C kept in freeze for 10 hrs. Charged in autoclave and heat 60-65 0 C for 24 hrs. Quenched the reaction mass in MDC and 30 % NaOH solution at RT and stirred for 15 minutes. Separated both organic and aq. Layer and deg. the org. layer. For stability concern oily mass obtained which was treated with conc. HCl to give N, N-Dimethyl ethanimidamide hydrochloride and its mechanism of formation is discussed in **scheme V.**

Anal. Calc. for $C_4H_{10}N_2$, Mol. Wt.: 86.1356, C: 55.78; H: 11.70; N: 32.52, **^{1}H NMR:** 400 MHz, DMSO: δ ppm: 2.278(s, 3H, -CH_3), 3.073(s, 3H, -CH_3), 3.121 (s, 3H, -CH_3), 8.663 (s, 1H, -HCl), 9.525 (s, 1H, -NH). **IR ν(cm1):** 650, 740.6, 790.76, 898.76, 941.19, 1043.43, 1056.91, 1074.27, 1109, 1193.85, 1219, 1261.35, 1277, 1381, 1420, 1441, 1466, 1510.15, 1570, 1626, 1633.6, 1676, 1686, 1794, 1859, 2129, 2972, 3072, 3367.47, 3400.25, **GCMS Mass** m/z: 86.

CONCLUSION

During synthesis process developed to produce safe and high purity Iguratimod API and consequent forced degradation studies total of four impurities are observed. Imp-I i.e methyl derivative of IGRD was formed when traces of un-reacted methyl iodide from N-2 stage reacted with IGRD. Use of strong acid/base and temperature control are critical during acid-base purification; otherwise it can form Imp-II and III. At last Imp-IV is formed due interaction with a reaction byproduct dimethyl amine and acetonitrile during acid-base purification.

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