

Synthesis and Characterization of Specified Impurities of Aceclofenac

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Received 23 December 2012 / Revised 23 January 2013 / Accepted 17 February 2013

Abstract: The present study aimed at synthesizing the process related potential impurities of aceclofenac. Aceclofenac is an orally administered phenyl acetic acid derivative with effects on a variety of inflammatory mediators. Process related impurities of aceclofenac listed in British Pharmacopoeia have been synthesized by modified methods and characterized by FT IR, MS and ¹H NMR data. Impurity A: [2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid (diclofenac) was synthesized by acid hydrolysis of diclofenac sodium; Impurity B: methyl [2-[(2,6-dichlorophenyl) amino] phenyl]acetate (methyl ester of diclofenac); Impurity C: Ethyl [2-[(2,6-dichlorophenyl) amino] phenyl]acetate (ethyl ester of diclofenac); Impurity D: methyl [[[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxy]acetate (methyl ester of aceclofenac) and Impurity E: ethyl [[[2-[(2,6-dichlorophenyl) amino]phenyl] acetyl] oxy]acetate (ethyl ester of aceclofenac) were synthesized by simple and convenient direct methylation and ethylation of diclofenac and aceclofenac respectively instead of tedious esterification process. Impurity F: benzyl [[[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxy] acetate (benzyl ester of aceclofenac) was synthesized by condensation of diclofenac sodium with benzylbromoacetate. Impurity I: 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one was synthesized by acid cyclization of diclofenac. The present study has provided an efficient method for synthesis of process related aceclofenac impurities.

Keywords: Aceclofenac, Impurity, Synthesis, Characterization

Introduction

Aceclofenac is an orally administered phenyl acetic acid derivative with effects on a variety of inflammatory mediators. Aceclofenac is chemically 2-[[[2-[(2, 6-dichlorophenyl)amino] phenyl]acetyl]oxy]acetic acid¹. Aceclofenac has been shown to have potent analgesic and anti-inflammatory activities, similar to indomethacin and diclofenac and due to its preferential cyclooxygenase-2 (COX-2) blockade, it has better safety than conventional non-steroidal anti-inflammatory drugs (NSAIDs) with respect to adverse effects on gastrointestinal and cardiovascular system. It is indicated for long term management of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It is also widely used in condition of mild to moderate pain and post-operative pain, dysmenorrhoea, musculoskeletal trauma and gonalgia.

Impurity is any component of a drug substance (excluding water) that is a chemical entity other than the active pharmaceutical ingredient². According to 'International Conference on the Harmonisation (ICH) guidelines impurities can be broadly classified into three categories for the drug substance produced by chemical synthesis^{3,4}.

- Organic impurities - starting material, process related products, intermediates and degradation products.
- Inorganic impurities – salts, catalyst, ligands and heavy metals or other residual metals.
- Residual solvents – organic and inorganic liquids used during production or crystallization.

The presence of impurities on pharmaceuticals can have significant effect on their quality and their safety. The safety of a drug is determined by its pharmacological-/toxicological profile as well as the adverse effects caused by the impurities in bulk and dosage forms. The impurities in drugs often possess unwanted pharmacological or toxicological effects by which any benefit from their administration may be outweighed. Therefore, it is quite obvious that the products intended for human consumption must be characterized as completely as possible⁵. The quality and safety of a drug is generally assured by monitoring and controlling the impurities effectively. Hence testing for impurities and their evaluation have been important elements of the International Conference of Harmonization (ICH) process⁶.

A description of the identified and unidentified impurities present in new drug substance is known as impurity profile. Regulatory requirements for the identification, quantification and control of impurities in drug substances and their formulated products are now being increasingly explicitly defined, particularly through the International Conference of Harmonization.

The most critical aspect of the elaboration of the guidelines was the definition of the levels of impurities for identification and qualification. Qualification is the process of acquiring and evaluating data for establishing the biological safety of an individual impurity or a given impurity profile at the levels specified. The level of any impurity present in a new drug substance that has been adequately tested in safety and clinical studies is considered qualified.

Chemistry and safety aspects of impurities are a central focus of this quality concept during the development stages. In the early drug development stages, the level of impurities is usually higher, experience and information are limited, and there is greater variability in the synthetic process, particularly during optimization and scale up⁷. Another important role of the synthesized impurities is its use as an impurity standard in the course of final step of impurity profiling, when selective, quantitative method is developed for determining the impurity⁸.

Keeping in view the stringent purity requirements from the regulatory authorities that the impurities $\geq 0.1\%$ must be identified and characterized. This present work describes synthesis of process related impurities of aceclofenac listed in British Pharmacopoeia⁹ (BP) and characterization of synthesized impurities by FT-IR, MS and ¹H NMR data

Experimental

Melting points were determined in open capillary tubes on a ThermoNik melting point apparatus and were found uncorrected. Infra red spectra were recorded on Fourier Transform IR spectrophotometer (Shimadzu FT-IR 8700) using KBR (ν_{\max} in cm^{-1}) disc method. ¹H NMR spectra were recorded in CDCl₃ on Bruker 200 MHz NMR spectrophotometer (chemical shifts in δ ppm) using TMS as an internal standard. The mass spectra were recorded on Shimadzu 2010A LCMS spectrophotometer (m/z and relative intensity). All the reactions were routinely monitored and purity of the compounds was ascertained by thin layer

chromatography on aluminium plates precoated with silica gel G (Merck silica gel 60 F₂₅₄) in various solvent systems of different polarity. All the chemicals (Sigma Aldrich, Acrose, Hi-media) and solvents (Qualigens) used were of AR grade and solvents were purified by suitable methods. Diclofenac sodium and aceclofenac were obtained from Karnataka Antibiotics & Pharmaceuticals Ltd., (KAPL) Bangalore as a gift samples. Aceclofenac impurities were prepared by following procedure

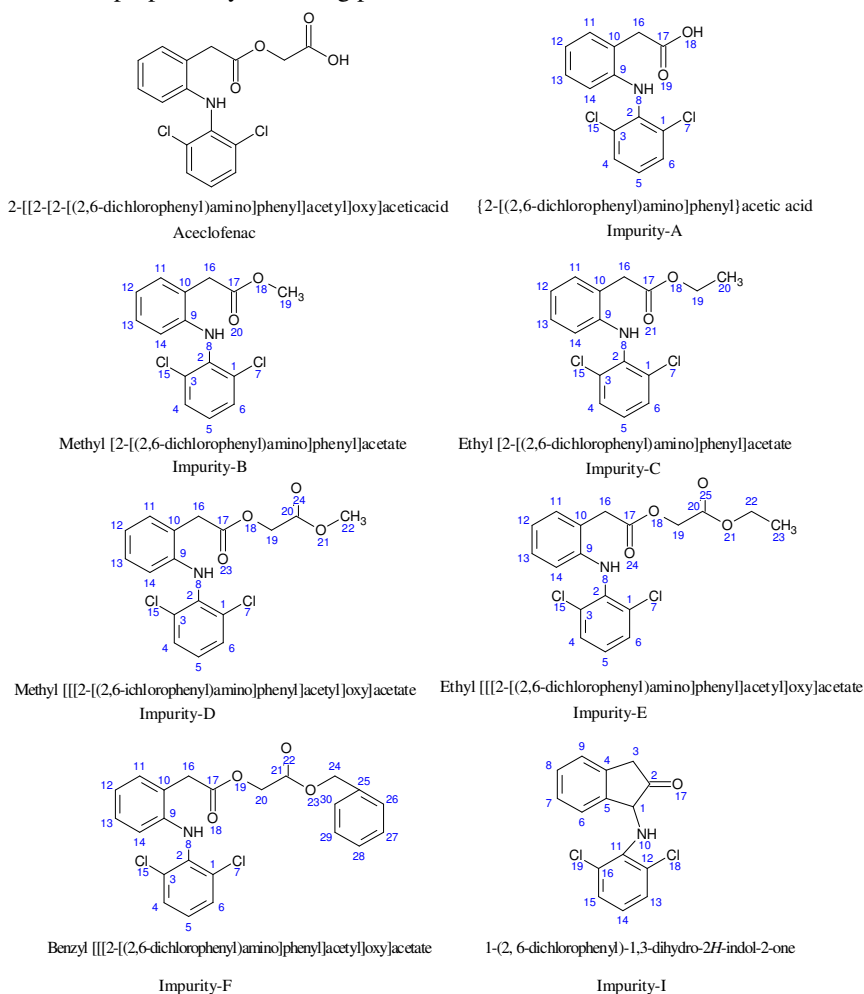


Figure 1. Structure of aceclofenac and specified impurities of aceclofenac synthesized

Synthesis of impurity - A: [2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid

5 g of Sodium 2-[(2,6-dichloroanilino)phenyl]acetate (diclofenac sodium) was taken in a 250 mL round bottom flask, 50 mL of 10% hydrochloric acid was added. The mixture was refluxed for 2 h in order to complete the hydrolysis of sodium salt. The reaction mixture was neutralized with dilute sodium carbonate solution. The mixture was filtered to get white solid and dried. The solid was recrystallized with methanol and water mixture (3:1) to get [2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid¹⁰ (Impurity-A).

Synthesis of impurity – B: Methyl [2-[(2, 6-dichlorophenyl)amino]phenyl]acetate

To a solution of 5 g of [2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid (0.0169 mol) in 80 mL of anhydrous *N,N*-dimethylformamide, 2.58 g of anhydrous potassium carbonate (0.0186 mol) and then 2.9 mL of dimethyl sulfate¹² (0.02535 mol) were added at room temperature. The reaction mixture was stirred for 12 h. The reaction mixture was filtered and washed with *N,N*-dimethylformamide. The combined filtrate was poured slowly into ice cooled water with continuous stirring to give white precipitate. The mixture was filtered and precipitate thoroughly washed with cooled water. The dry product was recrystallized from petroleum ether to give methyl [2-[(2, 6-dichlorophenyl) amino]phenyl]acetate¹¹(Impurity-B).

Synthesis of impurity - C: Ethyl [2-[(2, 6-dichlorophenyl) amino]phenyl]acetate

To a solution of 5 g of [2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid (0.0169 mol) in 80 mL of anhydrous *N,N*-dimethylformamide, 2.58 g of anhydrous potassium carbonate (0.0186 mol) and then 3.3 mL of diethyl sulfate¹² (0.02535 mol) were added at room temperature. The reaction mixture was stirred for 12 h. The reaction mixture was filtered and washed with *N,N*-dimethylformamide. The combined filtrate was poured slowly into ice cooled water with continuous stirring to give white precipitate. The mixture was filtered and precipitate thoroughly washed with cooled water. The dry product was recrystallized from petroleum ether to give ethyl [2-[(2, 6-dichlorophenyl) amino] phenyl]acetate¹³ (Impurity-C).

Synthesis of impurity - D: Methyl [[[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate

To a solution of 5 g of [[[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid (0.0141 mol) in 80 mL of anhydrous *N,N*-dimethylformamide, 2.18 g of anhydrous potassium carbonate (0.01577 mol) and then 2.7 mL of dimethyl sulfate¹² (0.0212 mol) were added at room temperature. The reaction mixture was stirred for 18 h. The reaction mixture was filtered and washed with *N,N*-dimethylformamide. The combined filtrate was poured slowly into ice cooled water with continuous stirring to give white precipitate. The mixture was filtered and precipitate thoroughly washed with cooled water. The dry product was purified using *N,N*-dimethylformamide and water mixture without heating to give methyl[[[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl]oxy]acetate (Impurity-D).

Synthesis of impurity - E: Ethyl [[[2-[(2,6-dichlorophenyl) amino]phenyl]acetyl]oxy]acetate

To a solution of 5 g of [[[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid (0.0141 mol) in 80 mL of anhydrous *N,N*-dimethylformamide, 2.18 g of anhydrous potassium carbonate (0.01577 mol) and then 2.8 mL of diethyl sulfate¹² (0.0212 mol) were added at room temperature. The reaction mixture was stirred for 18 h. The reaction mixture was filtered and washed with *N,N*-dimethylformamide. The combined filtrate was poured slowly into ice cooled water with continuous stirring to give white precipitate. The mixture was filtered and precipitate thoroughly washed with cooled water. The dry product was purified using *N,N*-dimethylformamide and water mixture without heating to give ethyl[[[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl]oxy]acetate (Impurity-E).

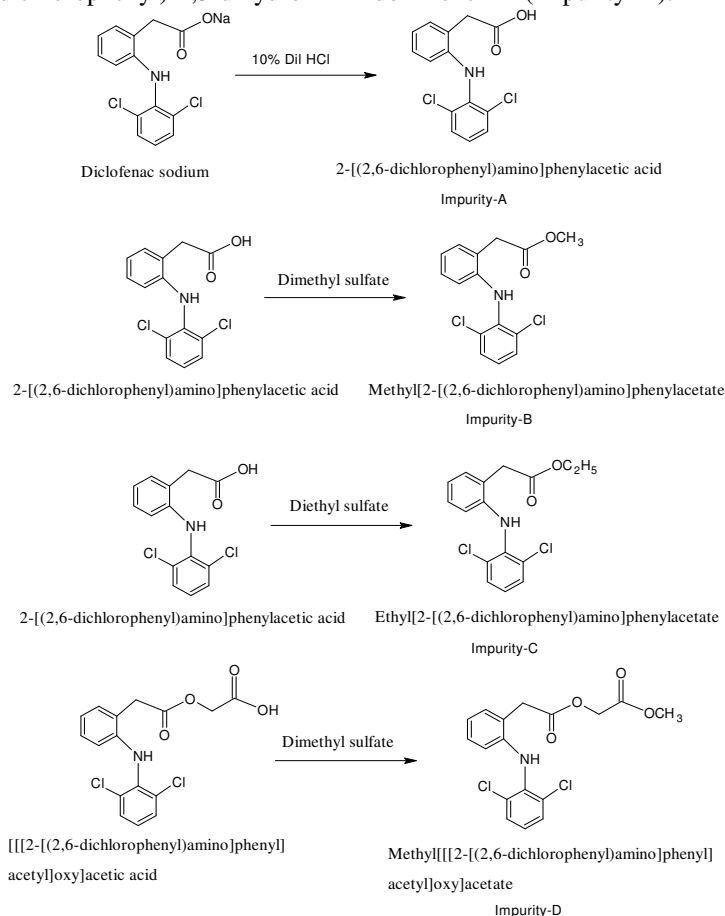
Synthesis of impurity-F: Benzyl [[[2-[(2,6-dichlorophenyl) amino]phenyl]acetyl]oxy]acetate

5 g (0.01571 moles) of sodium 2-[(2,6-dichlorophenyl)amino]phenylacetate were dissolved in 60 mL of *N,N*-dimethylformamide under heating to 50 °C and 6.89 g (0.017 moles) of

benzyl bromoacetate were added drop wise. The resulting mixture was stirred under 50 °C for 12 h. Then, the reaction solvent was removed under reduced pressure and sodium salts were precipitated by adding 400 mL of ethyl ether. The ether phase was then filtered and washed three times with 10 mL of water and then dried on sodium sulphate. The ether phase was then concentrated until an oil was obtained, and was washed twice with 20 mL of hexane. The resulting product was isolated by column chromatography using silica gel and petroleum ether: ethyl acetate (10:1) to give benzyl 2-[(2,6-dichlorophenyl)amino] phenylacetoxyl acetate¹⁴ in the form of white crystals.

Synthesis of impurity-I: 1-(2, 6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one

3 g of {2-[(2,6-Dichlorophenyl)amino]phenyl}acetic acid(impurity-A) in a 250 mL beaker and 50 mL of 25% sulfuric acid was added. The mixture was irradiated in micro-wave oven at 490 watts for 10 min. The reaction mixture was allowed to cool and reaction mixture was neutralized with 10% sodium carbonate solution. Then resultant solution was extracted with chloroform (4x10 mL). The chloroform extracts were combined and concentrated the organic solution gave 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one. The dried product was chromatographed in silica gel column using cyclohexane and ethyl acetate (3:1) gave pure 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one¹⁵⁻¹⁷ (Impurity - I).



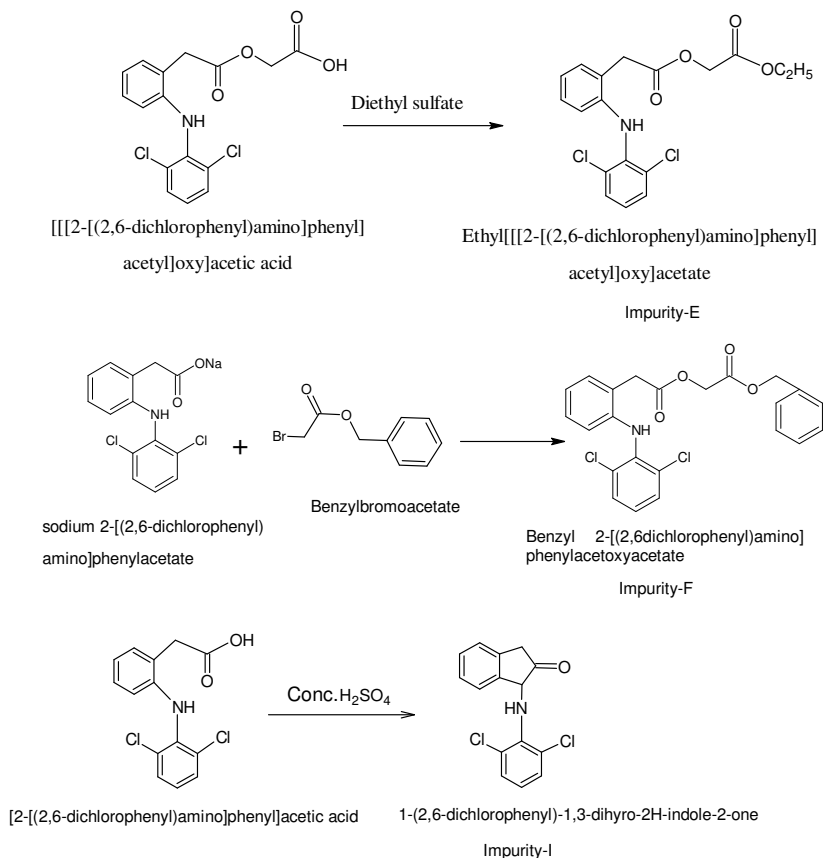


Figure 2. Reaction scheme for synthesis of aceclofenac impurities

Spectral data

Impurity-A: [2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid

IR (KBr, cm^{-1}): 3323.12(N-H str), 3074.12(C-H str), 1693.38 (C=O str), 1577.66, 1506.30 (C=C str), 1299.93 (C-O str), 1159.14 (C-N str), 939.27(C-Cl str) LCMS, mz^{-1} : 296.2.

Impurity-B: Methyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetate

IR (KBr, cm^{-1}): 3350.12(N-H str), 3012.60(C-H str), 1739.67(C=O str), 1583.45, 1450.37(C=C str-ar), 1259.43 (C-O str), 779.19, 748.33 (C-Cl str) $^1\text{H NMR}$ (CDCl_3 , δ ppm): 1.574(s, 3H, 19), 3.819 (s, 1H, 8), 3.752(d, 2H, 16), 6.568-6.529(d, 1H, 14), 6.947-6.917 (d, 1H, 12), 7.026-6.986(d, 1H, 13), 7.173-7.096(dd, 1H, 11), 7.258- 7.212(dd, 1H, 5), 7.368-7.328(d, 2H, 6,4) LCMS, mz^{-1} : 310.52.

Impurity-C: Ethyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetate

IR (KBr, cm^{-1}): 3296.12(N-H str), 3074.32(C-H str), 1710.74 (C=O str), 1577.66, 1452.30 (C=C str), 1236.29 (C-O str), 769.54, 760.14 (C-Cl str) $^1\text{H NMR}$ (CDCl_3 , δ ppm): 1.327-1.25(q, 3H, 20), 3.80(s, 2H, 16), 4.259(s, 1H, 8) 4.151(q, 2H, 19), 6.571-6.531 (d, 1H, 14), 6.953-6.915(d, 1H, 12), 7.016-6.975(d, 1H, 13), 7.167- 7.084(dd, 1H, 5), 7.255-7.214 (d, 1H, 11), 7.361-7.321(d, 2H, 4,6) LCMS, mz^{-1} : 324.33.

Impurity-D: Methyl [[[2-[(2, 6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate

IR (KBr, cm^{-1}): 3352.05 (N-H str), 3085.89 (C-H str), 1745.46, 1718.46 (C=O str), 585.38, 1454.23 (C=C str), 1228.57 (C-O str), 779.19, 744.47 (C-Cl str) $^1\text{H NMR}$ (CDCl_3 , δ ppm): 1.581(s, 1H, 8), 3.734 (s, 3H, 22), 3.94(s, 2H, 16), 4.688(s, 2H, 19), 6.577-6.538 (d, 1H,14), 6.695(s, 1H, 12), 6.978-6.940(d, 1H, 13), 7.021- 7.011(dd, 1H,11), 7.182-7.106 (dd, 1H,5), 7.358-7.250(d, 2H, 4,6) LCMS, mz^{-1} : 368.53.

Impurity-E: Ethyl [[[2-[(2, 6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate

IR (KBr, cm^{-1}): 3373.29 (N-H str), 3010.67 (C-H str), 1745.46 (C=O str), 1508.23, 1456.16 (C=C str-ar), 1207.36 (C-O str), 773.40, 750.36 (C-Cl str) $^1\text{H NMR}$ (CDCl_3 , δ ppm): 1.250-1.179(t, 3H, 23), 1.577(s,1H, 8), 3.937 (s, 2H, 16), 4.244-4.137 (q, 2H, 22),4.670 (s, 2H, 19), 6.574-6.534 (d, 1H,14), 6.724 (s, 1H, 12), 6.981-6.941(dd, 1H, 13), 7.022- 7.044 (d, 1H,11), 7.180-7097 (dd, 1H,5),7.359-7.260 (d, 2H, 4,6) LCMS, mz^{-1} : 382.60.

Impurity-F: Benzyl [[[2-[(2, 6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate

IR (KBr, cm^{-1}): 3377.12(N-H str), 3099.399(C-H-Ar str), 2960.53(C-H str), 1745.46 (C=O str), 1583.45, 1454.23 (C=C str-ar), 1508.23 (N-H bend), 1209.29 (C-O str), 740.61,771.47 (C-Cl str) $^1\text{H NMR}$ (CDCl_3 , δ ppm): 1.57 (CDCl_3), 3.93-3.87 (s, 2H, 16), 4.74-4.72 (s, 2H, 20), 5.22-5.17 (s, 2H, 24), 6.53-7.36 (m, 12H, Ar-H), 8.14 (s, 1H, NH) LCMS, mz^{-1} : 444.

Impurity-I: 1-(2, 6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one

IR (KBr, cm^{-1}):3087.82 (C-H str), 1730.03 (C=O str), 1230.21 (C-N str), 1164.92 (C-Cl str) $^1\text{H NMR}$ (CDCl_3 , δ ppm): 3.78 (S, 2H, 3), 6.42- 6.38(d, 2H, 6,7), 7.21 – 7.06 (m, 2H, 8,9), 7.41 – 7.33 (m, 2H, 13,14),7.54 – 7.49 (d, 1H, 14). LCMS, mz^{-1} : 279.

Results and Discussion

Physical data of synthesized aceclofenac impurities are presented in Table 1. Process related impurities of aceclofenac have been synthesized and the structure of synthesized impurities characterized by FT-IR, MS and $^1\text{H NMR}$ spectral data. Impurity-A was synthesized by simple and direct acid hydrolysis.

Table 1. Physical and analytical data of the synthesized aceclofenac impurities

Impurity	Mol. formula	Mol. wt	Yield, %	Melting point, $^{\circ}\text{C}$
Impurity-A	$\text{C}_{14}\text{H}_{11}\text{Cl}_2\text{NO}_2$	296.14	92	155-157
Impurity-B	$\text{C}_{15}\text{H}_{13}\text{Cl}_2\text{NO}_2$	310.17	95	110-112
Impurity-C	$\text{C}_{16}\text{H}_{15}\text{Cl}_2\text{NO}_2$	324.20	87	50-51
Impurity-D	$\text{C}_{17}\text{H}_{15}\text{Cl}_2\text{NO}_4$	368.21	78	108-110
Impurity-E	$\text{C}_{18}\text{H}_{17}\text{Cl}_2\text{NO}_4$	382.23	82	74-75
Impurity-F	$\text{C}_{23}\text{H}_{19}\text{Cl}_2\text{NO}_4$	444.30	85	70 -73
Impurity-I	$\text{C}_{14}\text{H}_9\text{Cl}_2\text{NO}$	278.13	85	124-125

Attempts were made to synthesize impurity B, C, D and E by sulfuric acid catalyzed direct esterification, but this method produced indole component as side reaction product due to presence of acid sensitive functional group in [2-[(2,6-dichlorophenyl)amino]phenyl acetic acid (diclofenac) and [[[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy] acetic acid (aceclofenac). Hence considerable modification was adopted to synthesize these impurities. Impurity B,C,D and E were synthesized by simple methylation and ethylation of

[2-[(2,6-dichlorophenyl)amino]phenyl acetic acid and [[[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy] acetic acid. These methods gave excellent yield of pure product. Impurity- F was synthesized by condensation of sodium 2-[2-(2,6-dichloroanilino)phenyl] acetate in *N,N'*-dimethyl formamide with benzylbromoacetate at mild condition and reaction gave excellent yield of impurity-F.

Impurity-I was synthesized by dehydration of impurity -A with concentrated sulfuric acid and impurity-A cyclized gave 1-(2,6-dichlorophenyl)-1,3-dihydro-2*H*-indol-2-one. Impurity-I contains indole nucleus and its formation was confirmed by the absence of N-H stretching peak in IR spectra.

Conclusion

The present study has provided efficient and modified method for synthesis of process related aceclofenac impurities. The characterized compounds shall be used as reference standard for developing HPLC method for their quantification in bulk drugs and pharmaceutical formulations.

Acknowledgments

Authors are thankful to Prof. B.G. Shivananda, Principal, Al-Ameen College of Pharmacy, Bangalore for providing necessary facilities. Authors also thankful to the Head of the department of inorganic chemistry, Indian Institute of Science, Bangalore and the Director, Astra Zeneca India Pvt. Limited, Bangalore for spectral analysis.

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