REVIEW ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Characterization of Impurities in Reverse Transcriptase Inhibitors by Profiling Techniques

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Abstract

There has been ever growing activity in impurities existing in pharmaceutical products as well as bulk drugs. According to a range of regulatory authorities, no longer solely purity profiles, however additionally impurity profiles, are now required. The technique facts for an individual impurity's organic safety are recognized as impurity profiling. The many developments in analytical viewpoints of impurity profiling of anti-retroviral (ARV) medicines and products used to deal with human immunodeficiency virus (HIV) infections are described in this review. ARVs work using inhibiting unique ranges of the viral contamination cycle to produce therapeutic benefits. Thus, drug classes are stratified as nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NTRTIs), non-NRTIs (NNRTIs), integrase strand transfer inhibitors, CCR5 antagonists, viral fusion inhibitors, and protease inhibitors (PIs). In this overview predominant focal point given on class that is reverse transcriptase inhibitors (RTIs). The wide variety of papers dealing with ARV drug impurity profiling is developing at an alarming pace. The cutting-edge overview article, which is based primarily on publications posted in the closing 15 years, tries to provide vast data concerning RTIs drug impurity profiling. RTIs which are labeled into two sub-categories, that is, NRTIs and NNRTIs. NRTIs pressure the HIV virus to use erroneous variations of building block, so contaminated cells cannot make more HIV and NNRTIs these are additionally referred to as "non-nukes." NNRTIs bind to a precise protein so the HIV virus cannot make copies of itself. The investigatory overview might also furnish the complete important points to the researchers who are working in the region of impurity profiling of RTIs. To the most tremendous of our information, no overview until date noted to center of attention on impurity profiling of RTIs.

Keywords: Anti-retroviral drugs, HIV, Impurity profiling, International council for harmonization guidelines, Reverse transcriptase inhibitors, HIV

Asian Pac. J. Health Sci., (2022); DOI: 10.21276/apjhs.2022.9.4S.06

Introduction

The primary intention of bulk drug industries and pharmaceutical industries is to produce the high-quality excellent product. The drugs are intended for saving lives in which even minute quantities of impurities are unacceptable. Hence, impurity profiling has obtain very importance.[1] The "International Council for Harmonization and Technical Requirements for Pharmaceuticals for use of Human (ICH)" gives the following definitions for impurities and impurity profile in new remedy substances.[2] An impurity is "Any a section of the new drug substance that is not the chemical factor characterized as the new drug substance." An impurity profile is "A depiction of the diagnosed and unrecognized impurities existing in a new drug substance." Analytical things to do for the detection, identification/structure elucidation, and quantitative determination of natural and inorganic impurities, as properly as residual solvents in bulk drugs and pharmaceutical formulations, are referred to as impurity profiling.[3] The value of drug impurity profiling is that it presents facts that can at once lead to drug therapy safety using decreasing impurity-related detrimental results of drug substances and preparations.[4] The pharmacological–toxicological profile of a drug, as nicely as the adverse outcomes brought about through impurities in bulk and dosage types, decide its safety, that is, the security of a drug product is decided no longer only by the toxicological properties of the active drug material itself, however additionally by means of the impurities that it contains. Another factor to think about is that the components need to keep its identity, strength, purity, and consistency all through the product's shelf life. Impurities in prescription drugs have to be monitored from the very starting, that is, from raw substances to the stop, that is, finished product, consisting of marketing survey.[5] The purpose of pharmaceutical impurity identification in the drug improvement phase is to completely elucidate the chemical structure of an unknown pharmaceutical impurity observed in both drug substances or drug merchandise above a particular threshold.[6]

According to current estimates, 34 million individuals global are dwelling with human immunodeficiency virus (HIV)/AIDS, with 2.5 million new infections taking place every year.[7] Infection-containing fluids, such as blood, breast milk, sperm, and vaginal secretions, are exchanged to spread the virus.[8-10] Sexual contact, injectable drug usage, transmission of contaminated physique fluids from mom to infant at some point of pregnancy, childbirth, or breast-feeding, and publicity of contaminated physique fluids to uncovered membranes or tissue are all feasible routes of viral infection.[10,11] Antiretroviral (ARV) remedy is the principal continue to be of HIV remedy and care, and it can drastically decrease HIV-related morbidity and mortality.[12-14] There are currently extra than

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How to cite this article: Shelke AV, Lokhande TN, Pardeshi V, Daware K, Jadhav S, Jadhav A. Characterization of Impurities in Reverse Transcriptase Inhibitors by Profiling Techniques. Asian Pac. J. Health Sci., 2022;9(4S):18-26.

Source of support: Nil

Conflicts of interest: None.

Received: 02/02/2022 **Revised:** 09/03/2022 **Accepted:** 30/04/2022

25 ARV agents authorized for HIV therapy through the United States Food and Drug Administration (US-FDA) in each single-and multi-drug formulations.[15]

There is a great need to review the analytical work reported so far in the literatures. Efforts have been made to collect the literature from 2005 up to the present (Figure 1).

Classification of Impurity[2]

Impurities can be Divided into the Following Categories

???

Sources of Impurities[17]

Impurities can also additionally be generated throughout the synthesis process, which requires quite a few intermediate steps between the beginning material and the completed product. Impurities can occur from a number sources, as shown in Figure 3.

The Following Methods can be used to Identify Impurities and Forced Degradant Products[18]

New drug improvement procedure necessitates the era of significant and dependable analytical facts at different stages of the process. A new pharmaceutical compound or medicinal drug must meet current purity requirements as a drug product to be viewed safe. These standards necessitate a responsive analytical method successful of measuring low levels of impurities. Impurity testing can be done using a number of approaches as shown in Figure 4.

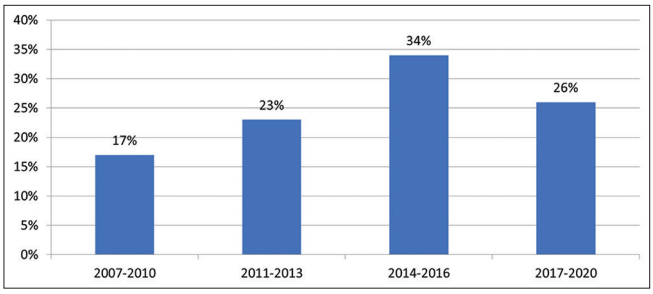


Figure 1: Year-wise publications for impurity profiling of reverse transcriptase inhibitors

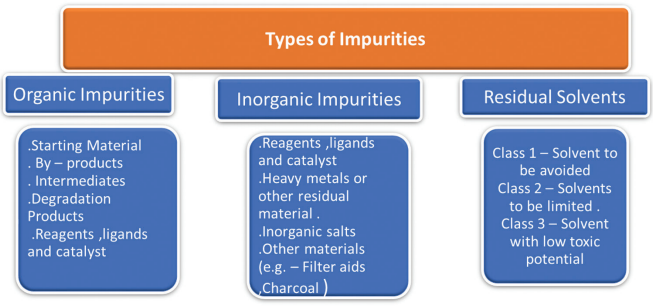


Figure 2: Types of Impurities

Regulatory Guidelines and Status on Impurity Profiling[19-26]

The ICH has issued guidelines for the validation of methods for evaluating impurities in new drug substances, products, residual solvents, and microbial impurities.[19] According to USP, the concepts of purity evolve over time and cannot be separable from analytical chemistry development.[20]

Impurities are Classified into Two Categories, According to BP

Qualified impurities

"Qualified impurities" are those that have been previously recognized as qualified by competent authorities.

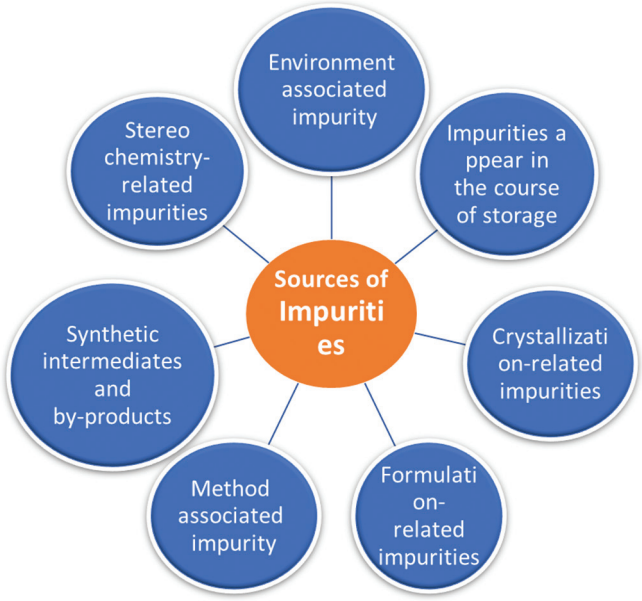


Figure 3: Sources of Impurities

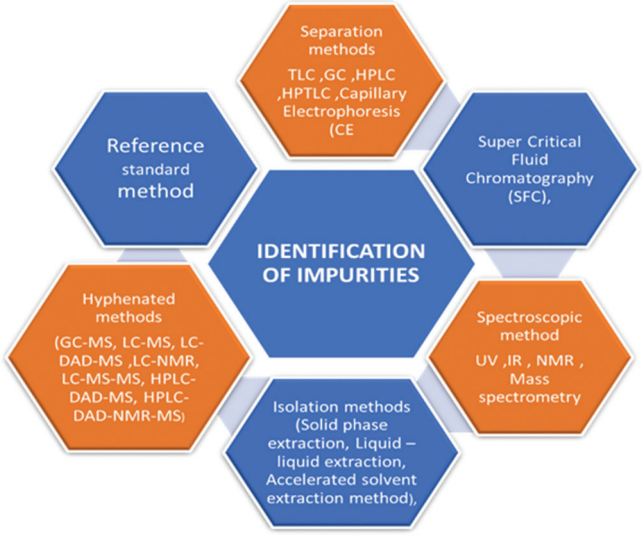


Figure 4: Impurity Testing by Various approaches.

Table 1: The acceptance criterion of impurity as per Indian Pharmacopoeia

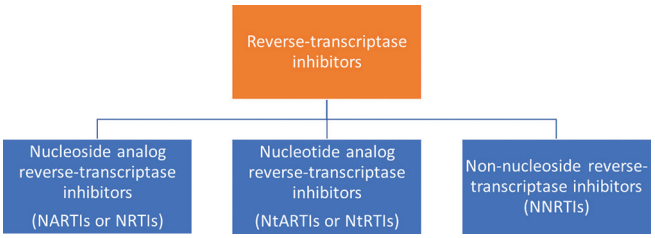
Criterion for drug	*Substances (%)*	*For Drug (%)*
Each identified specified Impurity	0.5	-
Each unidentified impurity	0.3	-
Total impurity	1.0	-
Each identified specific degradation product	-	1.0
Each unidentified degradation product	-	0.5
Total degradation product	-	2.0

Detectable impurities

"Detectable Impurities" are those that have not been found in any samples of the substances during the monograph's development or that occur in quantities <0.1% but have been shown to be limited by tests.[21]

The Indian Pharmacopoeia's acceptance criterion of impurity is provided in Table 1. The US-FDA has approved the guidance prepared under the guidance of the ICH.[22] The ICH guideline for impurities in pharmaceuticals was established by collaborative work of regulators and industry representatives from the European Union,[23] Japan,[24] and the United States[25] and it has helped to ensure that different regions have reliable requirements for the data that should be submitted to various regulatory agencies. The guidelines not only help sponsors of New Drug Applications or the FDA approved the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) as the first class of ARV medicines.

Reverse Transcriptase Inhibitors (RTI) come in Three Forms



The inclusion of these medications during RNA-dependent DNA or DNA-dependent DNA synthesis, which prevents the creation of either positive or negative strands of DNA, is an intriguing property of these drugs, for example, zidovudine, stavudine, lamivudine, didanosine, zalcitabine, emtricitabine (ETC), and abacavir. Non-NRTIs (NNRTIs) are the second class of RTI, for example, nevirapine, delavirdine, efavirenz (EFV) etravirine, and rilpivirine.[16] The growing demand for these agents stimulates a search for new even more effective drugs, but also calls for higher level of impurity control of these therapeutic substances and preparations so that they are in the highest possible degree free from any impurities that may come from the production process, as well as from decompositions products of active or auxiliary substances. Therefore, it seems appropriate to develop impurity profiling methods regarding their qualitative and quantitative analysis. For this aim, different analytical methods are developed for impurity profiling to determining anti-HIV drugs. One of the major group, that is, RTI from ARVs drugs are seem to in recent developments.

NRTI

NARTIs or NRTIs compose the first class of ARV drugs developed. To be included into the viral DNA, NRTIs need to be activated in the cell with the aid of the addition of three phosphate groups to their deoxyribose moiety, to shape NRTI triphosphates. This phosphorylation step is carried out by applying cellular kinase enzymes. NRTIs can result in mitochondrial impairment that leads to a wide variety of detrimental events, along with symptomatic lactic acidosis

Nucleoside analogue RTIs including zidovudine (ZDV), formerly AZT didanosine (ddI), lamivudine (3TC), stavudine (d4T), zalcitabine (ddC), and are used to treat HIV infections.

Zidovudine (AZT)

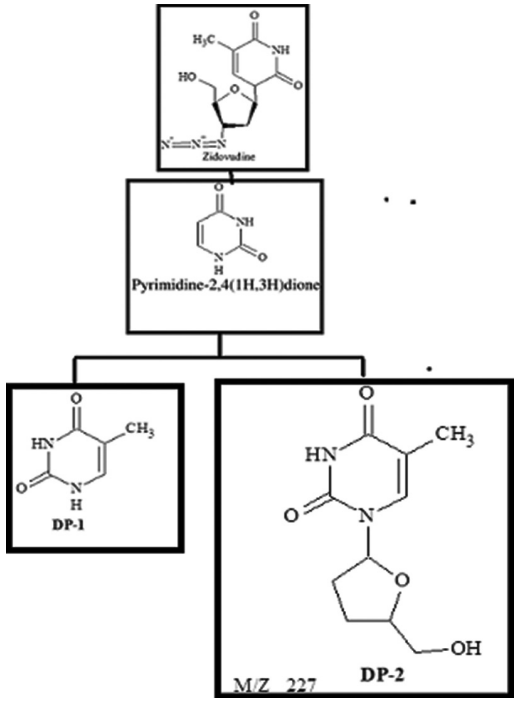
Zidovudine also known as AZT, ZDV, and azidothymidine, with a trade name Retrovir. Zidovudine was the first ARV drug which was approved by the FDA for treating HIV.

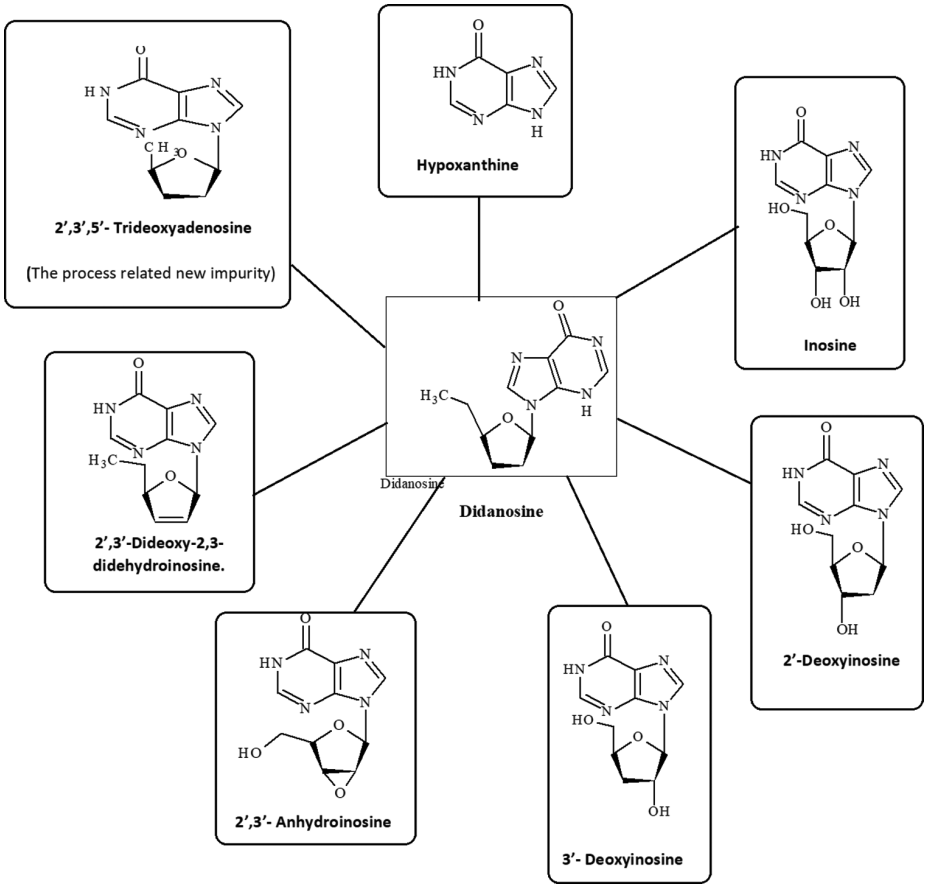
Vadgaonkar, et al. (2014)

Have described the synthesis of Zidovudine impurity in bulk and formulations, as well as the characterization of process-related impurities, and developed the isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) system, which has been optimized and validated according to ICH guidelines. The adduct impurity is formed due to condensation reaction. The intermediate was synthesized using a reflux reaction with Propionyl chloride and urea in a 1:1 ratio in the presence of ethanol as a catalyst. The synthesized process related impurity of Zidovudine (ZI) is Pyrimidine 2,4(1H,3H) dione (ZI). The amount of impurity in the bulk drug and formulation was found to be 0.057% and 0.0833%, respectively.[27]

Devrukhakar, et al. (2017)

Was study the degradation pattern of Zidovudine (AZT), researchers established a selective validated stability indicating LC/MS/MS assay process. Under acid degradation forced analysis, two





unknown degradation products, DP-1 and DP-2, were described and characterized using LC/ESI/MS/MS as of (m/z 127) and (m/z 227), respectively. DP-1 eluting at Rt 2.6 min while DP-2 eluting at Rt 12.0 min. The carcinogenic potential of both degradation products was predicted *in silico* toxicity profile using the TOPKAT program. In different models, the toxicity of degradation products was compared and estimated for AZT.[28]

Didanosine

Didanosine, also known ddI, which has trade names Videx and Videx EC, was the second FDA-approved ARV drug. It is an analog of adenosine.

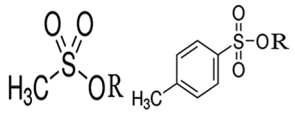
Srinivasa Rao, et al. (2007)

In HPLC study, six process-related known impurities and one unknown impurity were detected at concentrations ranging from 0.05 to 0.8%. One unknown impurity was consistently observed in HPLC during the laboratory preparation of didanosine, along with six known impurities, that is, hypoxanthine, inosine, 2-deoxyinosine, 3-deoxyinosine, 2,3- anhydroinosine, and 2,3-dideoxy-2,3-didehydroinosine. The process related new impurity in didanosine bulk drug was identified as 2,3,5-trideoxyadenosine.[29]

Lamivudine

Ramana et al. (2012)

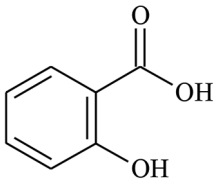
Has proposed two highly sensitive hyphenated techniques, namely, GC-MS and LC-MS for determining genotoxic impurity Alkyl Methane Sulfonates (AMSs) and Alkyl Paratoluene Sulfonates (APTSs) in lamivudine. Limit of quantitation for AMSs was found 1.5 μg/mL and for APTSs 1.0–1.5 μg/mL.[30]



Alkyl Methane sulfonate Alkyl Paratoulene sulfonate

Yellampalli et al. (2017)

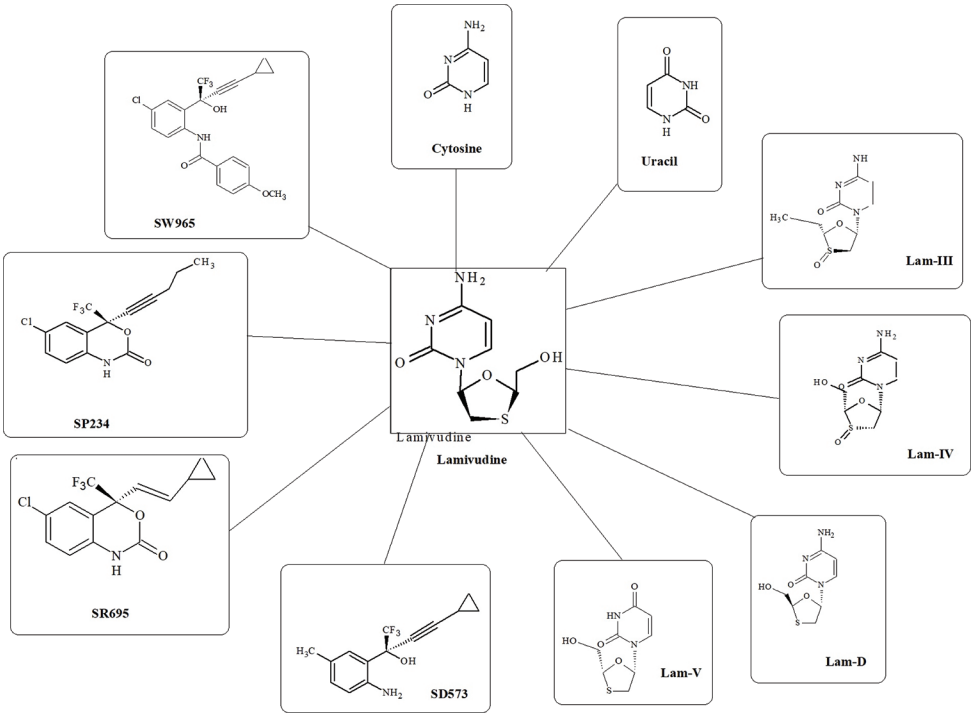
Has reported separation of Lamivudine, salicylic acid, and unknown impurities. Salicylic acid impurity (Rt = 14.88 min) was completely isolated from Lamivudine (Rt = 4.84 min). Salicylic acid recovery was found to be between 80 and 120%, with an RSD of 3.48%. The degrading impurities generated from force degradation studies are well separated.[31]



Salicylic Acid

Alexander et al. (2013)

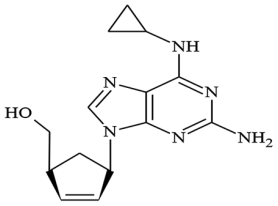
Has compared the impurity profiling of three ARV drugs in combination tablets containing Lamivudine, BMS-986001,



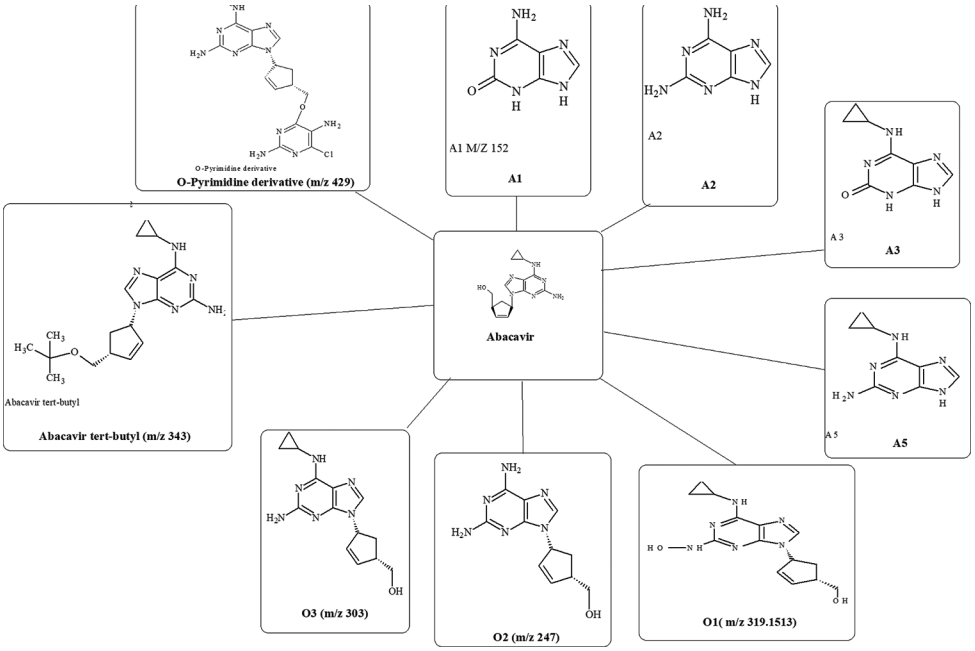
and EFV using supercritical fluid chromatography (SFC) and reverse phase liquid chromatography (RPLC). From these three APIs total 13 possible impurity/degradation products selected, all were resolved by RPLC namely Cytosine, Uracil, Lam-III, Lam-IV, Thymine, Lam-D, Lam-V, SD573, SR695, SP234, SW965, SE563, and SM097. By SFC 15, peaks were resolved. Lamivudine/Lam-D, Lamivudine/Lam-V, and EFV/SR695 were found to be the most difficult pairs to separate when it came to baseline separation. SFC was found to have the sensitivity at the 0.05–0.1 area % level when compared to RPLC.[32]

Abacavir

Abacavir, also known ABC, has the trade name Ziagen, is an analog of guanosine.



Abacavir



Rao et al. (2011)

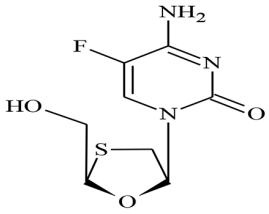
Has performed separation and characterization of abacavir sulfate forced degradation products using LC–MS/MS. Eight degradation products were developed, and their separation was achieved using an HPLC method. Abacavir sulfate, on acid hydrolysis, yielded five degradation Products A1, A2, A3, A4, and A5 which were characterized by molecular ion at m/z as A1 (152), A2 (151), A3 (192), A4 (Not Detected), and A5 (191). Three degradation products resulted from the oxidation of abacavir sulfate. The oxidation product O1 observed at (m/z 319.1513). O2 was observed at (m/z 247), O3 has fragment ion structures at (m/z 303).[33]

Satyanarayana et al. (2015)

Has performed **s**ynthesis and characterization of potent Abacavir impurities were performed. The silica gel was used for the column chromatography. The Agilent 1100 MSD spectrometer was used to record mass spectra in electro spray mode. On a 400-MHz Varian spectrometer, 1H NMR spectra were recorded. The synthesis and characterization of two possible Abacavir impurities, Abacavir tert-butyl (m/z 343), and O-Pyrimidine derivative (m/z 429), are described in this paper.[34]

ETC

ETC, also known as FTC, it has a the trade name Emtriva (formerly Coviracil). It is structurally similar to lamivudine and is approved for the treating HIV and also undergoing clinical trials for hepatitis B.



ETC

Pendela et al. (2010)

Has reported impurity profiling using LC–MS in a selected ETC sample in that total, nine peaks were investigated, with the majority of them being identified. By comparing their MS2 spectra to that of ETC, the unknown (UNK) impurities were identified. Peak 1 (m/z 262), Peak 2 (m/z 264), Peak 8 (m/z 265), Peak 3 (m/z 246), Peak 4 (m/z 230), Peak 5 (m/z 249), Peak 6 (m/z 364), Peak 7 (m/z 364), and Peak 9 (m/z 248).[35]

Kakadiya et al. (2011)

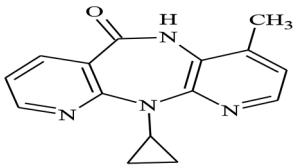
Have been identified Alkyl methanesulfonates as possible genotoxic impurities (GIs). For the determination of Methyl methanesulfonate and Ethyl methanesulfonate impurities in ETC, a sensitive LC/MS/MS method was developed and validated. For both analytes, the method had a very low limit of detection and limit of quantification of 0.3 g/g and 0.4 g/g, respectively. For both GIs, accuracy was found to be between 80% and 120%. MMS (m/z 110.9) and EMS (m/z 125.1) major fragments were found.[36]

NNRTI

NNRTIs are studied as the third class of ARV drugs. This class of drugs was first introduced at the Rega Institute for Medical Research (Belgium).

Nevirapine

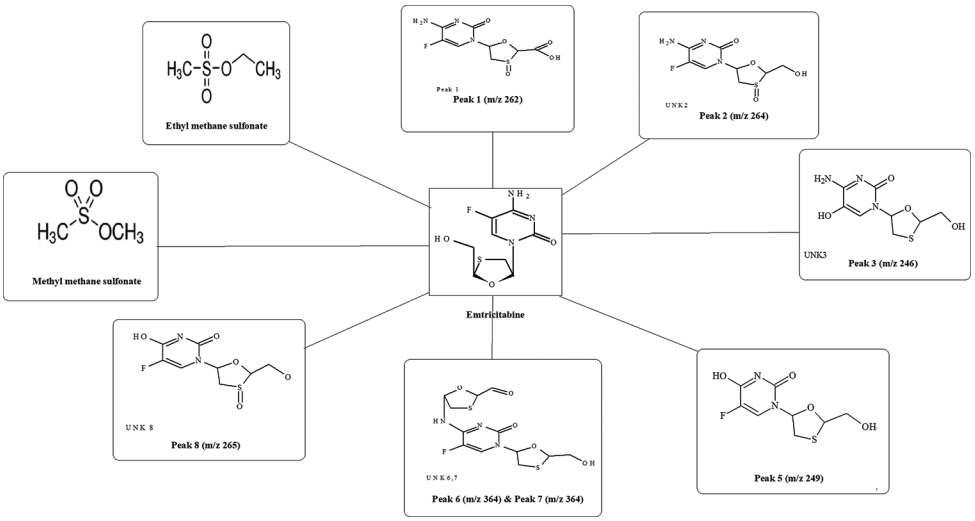
Nevirapine is recognized as trade name Viramune.

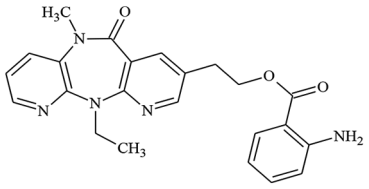


Nevirapine

Fenghe et al. (2009)

Was established the chemical structure of an unknown by-product Impurity 4 (m/z 419) produced during the synthesis of a nevirapine. A series of photo- and oxidative stress tests were used to figure out, where the impurity came from. This impurity is thought to



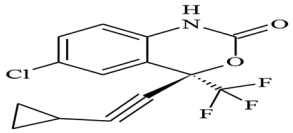


form as a result of a side reaction between the last intermediate and the oxidant used in the synthesis.[37]

Impurity 4 (m/z 419)

EFV

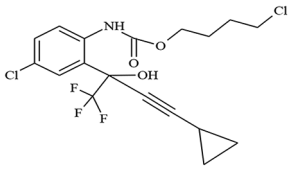
EFV has the brand names Sustiva, is an ARV medication used to treat and prevent HIV/AIDS.[1] It is generally applied with other ARVs. It may be applied for prevention and treatment of needle stick injury or other potential exposure. It is used in combination EFV/ETC/tenofovir.



EFV

Mulla et al. (2014)

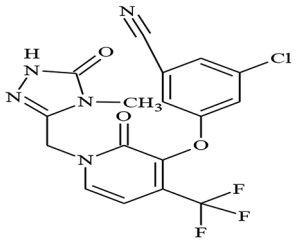
Has recorded the synthesis and isolation of an unknown in process impurity of EFV. A RP-HPLC was used to detect impurities in EFV ranging from 0.05% to 0.2% during the operation. Between 15.1 and 17.5 min, the EFV impurity fraction was obtained. The pure impurity (>95%) characterized and confirm the molecular structure of IUPAC name (4- Chlorobutyl) [4- Chloro-2(4-Cyclopropyl-1,1,1-Trifluro-but-3-yn-2-ol) Phenyl] Carbamate exhibited molecular ion at (m/z 421.8 amu).[38]



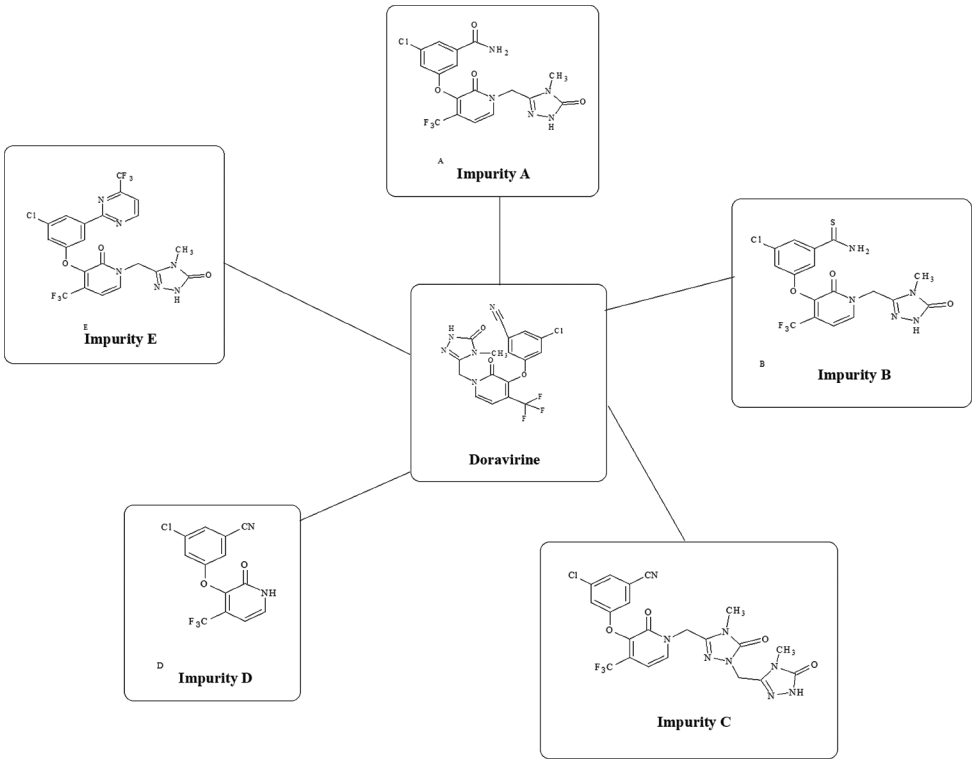
(4- Chlorobutyl)[4-Chloro-2 (4-Cyclopropyl -1,1,1-Trifluro-but-3-yn-2-ol)Phenyl]Carbamate (m/z 421.8 amu)

Doravirine

Doravirine (MK-1439), also called Pifeltro, is a non-nucleoside reverse transcriptase inhibitor invented by Merck and Co. and used in the treatment of HIV/AIDS. In August 2018, the FDA gave approval to doravirine**.** It is applied in combination with tablet as doravirine/lamivudine/tenofovir disoproxil fumarate (Delstrigo).



Doravirine



Zhang et al. (2016)

Has developed a quick and sensitive method for determining the structure of doravirine's major impurities. The thesis employs UHPLC-HRMS/MS techniques to determine the structure of unknown impurities. A total of five major trace-level impurities (A, B, C, D, and E) are reported in the UHPLC-UV chromatogram. The relative retention times of the five components Impurity A, B, C, D, and E were found to be 0.56, 0.83, 0.92, 1.10, and 1.35 min, respectively. According to the high-resolution LC/MS/MS data m/z of impurities are determined to be 443, 459, 536, 314, and 546 Da, respectively.[39]

Conclusion

From all above discussion, it is clear that impurities and related degradant products are the primary causes of poor medicine quality. Impurity profile of pharmaceuticals is receiving an increasing importance and drug safety receives more and more attention from the public and from the media. As a result, a critical activity in the drug production process is the evaluation, characterization, and quantification of all impurities and degradant products. In addition, the amount of impurities should be kept within regulatory limits, that is, within 0.1%.[40] Impurity profiling has progressed significantly. This review provides a perspective on impurities in drug substance and drug product of RTI. In this article, valuable information has been provided about the impurities types, various techniques of isolation and characterization, analytical techniques for the determination, quantification of impurities, and critical factors to be considered while preparation of the RTI APIs. These compiled data from past 15 years may of use for research for further studies in impurity profiling of RTI.

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