



A BRIEF REVIEW ON DIFFERENT ANALYTICAL TECHNIQUES FOR IMPURITY PROFILING IN ANTIVIRAL DRUGS.

Mahaparale Sonali Paresh¹, Patil Aishwarya Balu² Ahire Saurabh Nandkumar³
Associate Professor¹, M Pharm Research Scholar², M Pharm Research Scholar³

Pharmaceutical Quality Assurance¹, Pharmaceutical Quality Assurance²,
Pharmaceutical Quality Assurance³
Dr. D. Y. Patil College of Pharmacy Akurdi, Pune

❖ **Abstract:** -The Present overview article advocate and speak new technique of impurity profiling in Antiviral Drugs the usage of different analytical technique. The Drug formulations contain active pharmaceutical ingredients (APIs) and excipients. APIs present withinside the formulations contain a few undesired impurities, which influences purity of the APIs. Therefore, with along % purity, impurity profiling is also had to be done of all of the APIs. Impurity profiling has become a critical segment of pharmaceutical studies wherein each spectroscopic and chromatographic strategies discover applications. Antiviral drugs are a therapeutic class of drugs which have garnered interest the various public across the globe. High prescription and intake rates, seasonal top emissions because of common outbreaks of influenza, and critical spatial distribution in regions wherein a massive a part of the populace suffers from HIV are reported. And in recent times maximum antiviral pills used to deal with covid 19. The present overview covers diverse components associated with the analytical technique improvement for impurity profiling of an active pharmaceuticals in Antiviral drugs. Impurity profiling encounters issues in bulk drugs because it assists in improving bulk drug quality ultimately benefiting patient.

❖ **Key Words:** -Impurity Profiling, Antiviral Drugs, Chromatographic Conditions, ICH Limits.

❖ **Introduction:** - The bulk drug industry forms base of all pharmaceutical industries as it is the source of active pharmaceutical ingredients (APIs) of specific quality. The quality of bulk drug is important for pharmaceutical industries. Different quality control checks are performed to maintain quality and purity of bulk drugs. The pharmacopoeias specify not only purity but also puts limits which can be very stringent on levels of various impurities.[1]

Impurity may be defined as the substance which exists with original drug as an intermediate or a primary material that is formed due to any change in condition of reaction or a reactant. Basically, pharmaceutical impurities can be divided into three types.[2]

Impurity profiling is a general term including structure elucidation/identification as well as determination of the impurities of a chemical substance. The significance of this process in pharmaceutical research and development has been emphasized multiple times with different analytical techniques always being mentioned as a widely used and extremely valuable analytical tool in this field.[3]

Viruses have too simple a structure to multiply themselves. For multiplication, a virus invades a cell, using the biochemical mechanisms of this cell to make new viral proteins and genetic material. So, virus and host cell are intimately connected and an effective antiviral drug must be able to distinguish the virus from the host cell. In the last twenty years there has been a growing understanding of viral multiplication, which has allowed us to develop new drugs in the battle against viral infections. With that new drug development, the quality and purity of drug substances is important. Impurity profiling of Anti-viral drugs is an important part for development.[4]

❖ **Impurity Profiling:** -

- **Definition:** -
- **Impurity:** - Impurity is any unwanted material that affects the purity of material of interest i.e., active pharmaceutical ingredient or drug substance. An impurity is considered as any other organic or inorganic material, along with the drug substance, or ingredients, arising during synthesis or unwanted chemicals that remain with APIs.[1]
- **Impurity Profiling:** - Impurity profiling is a general term including structure elucidation/identification as well as determination of the impurities of a chemical substance. Impurity profiling of a substance under investigation is an process where describes the maximum possible types of identified or unidentified impurities present in any sample of APIs produced by a specific controlled production process.[2]
- **Need:** - Nowadays Antiviral drugs having a great approach for their effect on covid 19. Antiviral drugs are the important part of treatment of covid 19. So, the production and maintaining quality is very important task for pharmaceutical department. While carrying out production process of any formulation it is a pre-requisite to analyse the presence of impurities in the raw materials used for production. These impurities may interfere with physical and chemical properties of APIs. The presence of these unwanted substances or

unwanted chemicals may also affect safety parameters of drug by producing adverse drug reactions or toxicities in the body has compromising safety and efficacy of APIs. So, the impurity profiling is needed.[5]

- **Classification of impurities:** - Impurities are classified by the following ways. [6]
- Organic Impurities (process and drug- related).
- Residual solvents.
- Inorganic impurities.
 - **Organic Impurities:** - They are formed during manufacturing process or during the period of storage of drug substances can in APIs or drug product formulation. They can arise from intermediates, starting materials, degradation products, unintended by-products. They are volatile or non-volatile substances which may arise from contamination of one enantiomeric form with another or racemisation. This can lead to undesired biological activity. These days, genotoxic pharmaceutical impurities are becoming a matter of concern, which have ability to increase the risks of cancer in patients.
 1. Starting Materials or Intermediate Impurities: - If proper care is not taken in each step during the multistep synthesis of drug, these impurities are present in almost every API
 2. By-Products: - In the process of drug synthesis, getting a single end product with 100 % yield is very difficult, as the undesired product is always formed.
 3. Degradation Products: - During the manufacturing of bulk drugs impurities can be formed by degradation of the end product. Degradation during improper storage or due to aging of drug is also a major source of degradation.
 4. Synthesis Related Impurities: - During the process of synthesis, from raw material, solvent, intermediate, by product the completely new chemical entity are being generated. In the process of synthesis, if any impurity is present in trace or may be an insignificant amount in any of substance involved in the reaction, then it will finally result in the production of final product which is contaminated with one or more unwanted materials that can be called as impurity.
 5. Formulation Related Impurities: - Mostly suspensions and solutions are very prone to degradation due to hydrolysis. Water which is used in formulation plays an important role, it not just contributes to impurity but they also provide situation for catalysis and hydrolysis.
 6. Hydrolysis: - It refers to breakdown or degradation of drug products.
 7. Oxidative Degradation: - Drugs such as nitroso/ nitrile derivative, hydrocortisone, heterocyclic aromatic ring, degradation of hydrogen peroxide solution.
 8. Photolytic Cleavage: - The drug substances undergo cleavage during manufacturing or storage of drug when they are exposed to light.
 9. Decarboxylation: - It is the chemical reaction where carboxyl group is removed from a molecule.

- **Inorganic Impurities:** - By using various principle and pharmacopoeia inorganic impurities are detected. They are usually found during the process of manufacturing. The various inorganic impurities include:
 1. Reagent, Ligands and Catalysts These impurities occur very less during manufacturing. It will create a great problem if proper procedure is not followed.
 2. Heavy Metals Water is the main solvent used during manufacturing process and it can be the source of various heavy metals, such as cadmium, manganese, lead. demineralised water or glass lined reactors are used to avoid it
 3. Other Materials: - The various filters or the substance which are used for filtration, centrifuge bags used in manufacturing process also contribute to impurity.
- **Residual Solvents:** - They are volatile chemicals which are used during the process of manufacturing. Most these solvents are toxic and also cause environment hazard, and their complete removal is also very difficult. Residual solvents can be divided into 3 classes based upon the risks they can possibly cause to human health.[6]

➤ **Sources of impurities:-** From the preceding discussion, it is clear that impurities can originate from several sources; such as;

- a. Crystallization-related impurities,
- b. Stereochemistry-related impurities,
- c. Residual solvents,
- d. Synthetic intermediates and by-products,
- e. Formulation-related impurities,
- f. Impurities arising during storage,
- g. Method related impurity,
- h. Mutual interaction amongst ingredients,
- i. Functional group-related typical degradation
- j. Process-related drug substance
- k. Degradation drug substance or drug[7]



- **Conventional approaches for the isolation and characterization of impurities:** - Before two and half decades, there were no precise methods to isolate and characterize the impurities. The characterization of impurities was based on the quantification of active ingredient content by nonspecific titrimetric and photometric methods (such as UV spectroscopy, IR, Raman spectroscopy, and nuclear magnetic resonance [NMR], which were supported by the physical constants and some limit tests for known impurities. Even the pharmacopeia was suggesting various nonspecific characterization methods for determining the content of active ingredients. Due to the emergence of chromatography in mid-19th century, various chromatographic methods such as capillary electrophoresis (CE), Chiral separations, gas chromatography (GC), high-pressure liquid chromatography (HPLC), supercritical fluid chromatography, and Thin-layer chromatography (TLC) were employed for the separation of various impurities in drug substance and products. Followed by the isolation/enrichment/synthesis by solid phase extraction, liquid– liquid extraction, accelerated solvent extraction, supercritical fluid extraction, column chromatography, flash chromatography, TLC, etc., and later spectrophotometric characterization of impurities using UV spectroscopy, IR, Raman spectroscopy, and NMR[5]
- **ICH limits of impurities:** - Various regulatory authorities such as International Conference of Harmonization [ICH], United States Food and Drug Administration (USFDA), Canadian Drug and Health Agency, etc have given various specifications for impurities. Impurities in new drug substances and drug products are identified and estimated with new approaches to quantitation and qualification. Regulatory requirements for identification, quantitation and control of impurities in drug substances and their formulation are now being increasingly unambiguously defined, particularly through ICH. The ICH of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has published guidelines for validation of methods for analysing impurities new drug substances, products, residual solvents and microbiological impurities. According to ICH guidelines, if impurities are less than 0.1 %, identification of impurities is not considered to be necessary unless these are expected to be potent and toxic. APIs should include limits for organic impurities, residual solvents, and inorganic impurities. ICH has given following limits for impurities: When dose is less than 2 gm/day, impurity present should be less than 0.1 % or 1 mg/day intake, whichever is lesser. When dose is more than 2 gm/day, impurity present should be less than 0.05 % of intake.[5]
- ❖ **Antiviral Drugs:** - Antiviral drugs are a therapeutic class of medicines that have garnered interest among the public around the globe. High prescription and consumption rates, seasonal peak emissions due to frequent outbreaks of influenza, and important spatial distribution in areas where a large part of the population suffers from HIV are reported. And nowadays most antiviral drugs used to treat covid 19.[2]

- **Mechanism of action of antiviral drugs:** -
 1. Attachment vaccination, immunoglobulins, inosiplex (interferons)
 2. Penetration (amantadine, rimantadine, tromantadine)
 3. Uncoating (amantadine, rimantadine, tromantadine)
 4. Replication - RNA synthesis (amantadine, rimantadine, tromantadine) foscarnet, ribavirin –
 5. DNA synthesis idoxuridine, trifluridine, acyclovir, ganciclovir, foscarnet zidovudine
 6. Viral protein synthesis (interferons)
 7. Assembly metasizon (interferons)
 8. Release (interferons)[4]

- **Characterization of impurities:** - Once an impurity is detected, it is necessary to estimate or quantify it. Initial estimation of impurities present in the new drug molecule involves use of reference standard API. Generally, both chromatographic and non-chromatographic techniques are used for isolation of impurities prior to its characterization. Most commonly used separation techniques involved are: -
 - **Accelerated Solvent Extraction Method (ASE)** :-ASE is the technique used to carry out extraction process at a faster rate. ASE provides extraction of impurities, is very fast as compared to Soxhlet extraction or sonication.[5]
 - **Supercritical Fluid Extraction (SFE):** - A substance at a temperature or pressure above its critical point behaves as a supercritical fluid which can diffuse through materials like gas, and dissolves solids like a liquid. It has very fast and gives high rate of extraction. and is most preferred and effective solvent for unstable compounds. Supercritical fluid chromatography (SFC) can be used on an analytical scale, where it combines many advantages of high-performance liquid chromatography (HPLC) and gas chromatography (GC).
 - **Column Chromatography:** - Column chromatography is based on principle of partition chromatography and works under influence of mobile phase involving separation of components of the sample during its passage through stationary phase. This is especially useful if one is trying to separate and purify a reaction mixture preparing an intermediate in a sequence of reactions. The corresponding disadvantage is column may take a long time to properly prepare and use.[5]
 - **Thin Layer Chromatography (TLC):** - TLC is the technique used for the identification of various components up to trace amounts. This technique has been used for developing stability-indicating analytical method. The detection using TLC is based upon the chemical reaction between the components and detection reagent. TLC is very much used during initial degradation and stress studies to study the number of degradation products formed.[5]

- **Gas Chromatography (GC):** - GC is used as a technique for qualitative and quantitative estimation of APIs, particularly with regards to detection of impurities which are volatile and thermo-stable in nature. It can be used as a limit test for solvent residue and other volatile impurities in drug substances. It is also utilized for characterization of raw materials used in synthesis of drug molecules.[5]
- **High Performance Liquid Chromatography (HPLC):**- HPLC is a versatile method of analysis as it is not limited to volatile or stable sample and separation is based on the fact that certain compounds have different migration rates on a particular stationary and mobile phase. HPLC also involves monitoring of stability of pure drug substance and in case of drug formulations. It can be applied for quantification of degradation products as well as impurities.[5]
- **Flash Chromatography:** - Flash chromatography is air pressure driven hybrid of medium pressure and short column chromatography. It is used to speed up the flow of solvent, which dramatically decreases the time needed to purify sample.
- **Capillary Electrophoresis (CE):** - Electrophoresis separates charged molecules by migrating them towards opposite poles on the basis of their physical characteristics. Impurities which are too polar in nature to give sufficient retention with RP-HPLC are easily separated by CE. It is established as a powerful tool for separation of charged drugs, impurities.
- **Micellar electrokinetic chromatography (MEKC):** - a separation mode of capillary electrophoresis (CE), has enabled separation of electrically neutral analytes.
- Various other instrumental methods are also used for the purpose of characterization of impurities in any sample. These methods include:
 - **NMR Spectroscopy:** -The versatility of nuclear magnetic resonance (NMR) spectroscopy has made it an inevitable tool for determination of chemical structure. NMR spectroscopy which is used to study chemical bonding, advanced two or multi-dimensional NMR spectroscopy such as Correlation Spectroscopy (COSY), Nuclear Overhauser Enhancement Spectroscopy (NOESY), Heteronuclear Correlation Spectroscopy (HETCOR), Incredible Natural Abundance Double Quantum Experiment (INADEQUATE) are developed for determination of complex structure of molecules. Time domain NMR spectroscopy is used to study molecular dynamics in solutions

Advantage: NMR can detect very fine structural components, qualitative and quantitative determination of organic and inorganic molecules, Versatile technique.

Disadvantage: - Time consuming, it is very expensive, long time take for interpretation.

- **Mass Spectroscopy (MS):** -In the past several decades, MS has emerged out as an inevitable tool for carrying out characterization of impurities present in pharmaceutical products.

Advantage: - Small sample size is required., It is fast technique, also differentiate isotope with GC and LC.

Disadvantages: - The cost, requiring a significant materials/equipment budget. Multifunctional systems do not exist.

- **UV-Visible Spectroscopy:** -UV-VIS spectroscopy is based on the absorption of visible and ultraviolet (UV) radiation in the wavelength range of 200-800 nm. Electrons in the bond within molecule become excited to occupy a higher quantum state by absorbing energy passing through solution. Every molecule has its own absorption maxima. UVVIS spectroscopy is one of the easiest methods to determine purity of drug substance. UV-VIS spectroscopy can only be used with those samples where component or some of its derivatives are spectrophotometrically active. UV-VIS spectroscopy is also used as stability-indicating method for characterisation of impurities or degradation products formed.

Advantages: - very cost effective, it is non-destructive. quantitatively and qualitatively on pure substances.

Disadvantages: - limited for molecules which absorb uv radiation.

- **IR Spectroscopy:** - IR spectroscopy provides a complex but unique fingerprint of any molecule which helps in analysing drug samples and determining presence of impurities in drugs. It can also be used for determining presence of polymorphs of drugs. Photoacoustic spectroscopy in infrared region is a helpful technique to characterize impurities in pharmaceutical products. Sample is subjected to electromagnetic radiation ranging between 500 cm^{-1} and 4000 cm^{-1} . wavelengths absorbed are characteristic for various kinds of bonds which help in determining structure of samples.

Advantages: - It is cost effective as compared to NMR. Fast interpretation

Disadvantages: - Detailed information cannot give, limited to IR active compounds.

- **Hyphenated Techniques:** - Hyphenated techniques involve highly sophisticated instrumentation consisting of two or more instruments configured together with help of well advanced and efficient interfaces. Such as mass spectrometers are attached to a GC or HPLC, are inevitable tools in identification of minor components such as drugs, impurities, degradation products. Hyphenated technique like HPLC-NMR or HPLC-MS are very efficient hyphenated techniques but due to high cost, high running expenses and high time consumption, PDA detectors attached to HPLC are better alternative. UVHPLC method to determine impurity profile can be used only when impurity is spectrophotometrically active.[5]

Drug		Technique	Chromatographic Conditions		Performance			Ref
			Column & Detector	Mobile Phase	LOD	LOQ	% Recovery	
1.	Valacyclovir [Imp- F, Imp- G]	RP-HPLC	C 18	Acetonitrile: Methanol	0.0024 0.04	0.0082 0.136	99.9 % 103.2 %	9
2.	Efavirenz	RP-HPLC	C 18	Methanol: Water	0.1	0.3	-	10
3.	Zidovudine	RP-HPLC	C 18 UV 270 nm	Ammonium acetate buffer: Acetonitrile (75:25)	-	-	-	10
4.	Lamivudine	RP-HPLC	C 18 UV 270 nm	Ammonium acetate buffer: Acetonitrile (75:25)	-	-	-	10
5.	Nevirapine	RP-HPLC	C 18 UV 283 nm	Acetonitrile: Phosphate Buffer (65:35)	-	-	-	11
6.	Stavudine	RP-HPLC	C 18	Ethyl acetate: Methanol: Toluene: Ammonia (38.7:19.4:38.7:17)	-	-	-	11
7.	Amprenavir Atazanavir Darunavir Lopinavir Ritonavir	RP-HPLC	C 18 UV 280 nm	Acetonitrile: 0.05 % Formic Acid	-	-	-	11
8.	Dolutegravir	RP-HPLC	C 18 UV 240 nm	0.1% Trifluoroacetic acid in water: Methanol	0.005	0.015	R ² =0.998	12
9.	Lopinavir	RP-HPLC	C 18 PDA	0.02M KH ₂ PO ₄ : Acetonitrile	0.028	0.084	90-100%	13
10.	Entecavir	RP-HPLC	C 18	Ammonium phosphate buffer with Glacial acetic acid: Acetonitrile	0.014	0.035	R ² =1	14
11.	Sofosbuvir	RP-HPLC	C 18	Water with 0.2% Formic acid: Acetonitrile	0.07	0.36	93%	15

12	Ritonavir	RP-HPLC	C 18 PDA	Methanol: Acetonitrile: Water (60:20:20)	0.026 1	0.079	92%	16
13	Ombitasvir Paritaprevir Dasabuvir	RP-HPLC	C 18	Phosphate Buffer (PH 7): Acetonitrile (35:65)	-	-	R ² =0.999	17
14	Efavirenz Acyclovir	RP-HPLC	C 18	Methanol: Water	-	-	-	10
15	Amantadine	RP-HPLC	C 18	Methanol: Water	-	-	-	10
16	Valacyclovir V1 Guanine V2 Acyclovir V3 Unknown	RP-HPLC	Daicel chiral PDA 254nm	0.1% Phosphoric acid: Methanol (90:10)	-	-	100%	18
17	Zanamivir Amantadine	UPLC ESI MS/MS	C 18 BEH	Water: Acetonitrile (0.1% Formic acid)	0.1- 0.4	0.2- 1.3	63%	10
18	Laninamivir Peracivovir	UPLC ESI MS/MS	C 18 BEH	Water: Acetonitrile (0.1% Formic acid)	0.1- 0.2	0.2- 0.7	70%	10
19	Peniciclovir Famciclovir Valacyclovir	UPLC ESI MS/MS	C 18 BEH	Water: Acetonitrile (0.1% Formic acid)	0.1- 0.2	0.3- 0.6	60%	10
20	Acyclovir	UPLC	C 18 BEH 254 nm	0.25% Formic acid: water	0.3	1		19
21	Abacavir	LC MS-MS	C 18	0.1 Formic Water: Methanol	2	12	100%	10
22	Atazanavir	LC MS-MS	C 18	0.1 Formic Water: Methanol	2	12	100%	10
23	Darunavir	LC MS-MS	C 18	0.1 Formic Water: Methanol	2	12	100%	10
24	Efavirenz	LC MS-MS	C 18	0.1 Formic Water: Methanol	2	12	100%	10
25	Endinavir	LC MS-MS	C 18	0.1 Formic Water: Methanol	2	12	100%	10

26	Lopinavir	LC MS-MS	C 18	0.1 Formic Water: Methanol	2	12	100%	10
----	-----------	----------	------	----------------------------	---	----	------	----

❖ **Conclusion:** - The present study may help the researchers in the selection of column, mobile phase, ionization technique, and analysers for the different analytical instrument to carried out impurity profiling. In conclusion, impurity profiling helps in conforming guidelines by regulatory authorities (ICH, USFDA etc.) regarding impurity levels in a drug. Impurity profiling is beneficial in deciding safety parameters for drugs. Various newer techniques are contributing major role in isolation and characterization of impurities present along with the drug. n for the structural elucidation of both known and unknown impurities. This review provides information on analytical strategies employed for the determination of various impurities in different antiviral drug. Most of the research work on impurity profiling includes the use of C-18 column and mobile phases containing 0.1–1% of formic acid. Based on the reviewed analytical data, it is concluded that in the field of antiviral drugs there are still crucial issues to be studied.

❖ **References:-**

1. Venkatesan P. and Valliappan K. Impurity Profiling: Theory and Practice, Journal of Pharmaceutical Science and research. ISSN 0975-1459 Vol. 6(7), 2014, 254-259
2. Anurag J., Sanjay S. Recent trends in impurity profiling of pharmaceutical products. MIT International Journal of Pharmaceutical Sciences .ISSN 2394-5338 (Print); 2394-5346 (Online). Vol. 3, No. 1, January 2017, pp. 1–6.
3. Phatangare M.D. ,Deshpande M.M , Sanap P.S.,Kachave R.S. ,Chavan M.J. RECENT TRENDS IN IMPURITY PROFILING METHODS USING ANALYTICAL TECHNIQUES.World Journal Of Pharmaceutical Research . ISSN 2277– 7105 Volume 9, Issue 12, 534-542
4. Wiltink EHH, Janknegt R. Antiviral drugs. Pharm Weekbl [Sci] 1991;13(2):58-69
5. Sunny Nagpal, Karan, Ashutosh Upadhyay, T. R. Bhardwaj and Arti Thakkar. A Review on Need and Importance of Impurity Profiling ISSN 1573-4129 2011, 7, 62-70
6. Dipankar N. Bidhya S. Impurity Profiling-A Significant Approach in Pharmaceuticals . Bentham Science Publishers . ISSN 1875-676X 2019, 15, 669-680
7. Warad T.A., Bhusnure O.G*, Gholve S.B Impurity profile of pharmaceuticals ingredient: a review . JOURNAL: JOURNAL OF PHARMACY RESEARCH 2016,10(7),523-533.
8. Gijriig S. Babjgk M. Balogh G. Brlik J. Csehi A. Drug impurity profiling strategies. ELSEVIER Talanta 44 (1997) 1517-1526
9. Prakash K. Baishakhi D. Nagiat T. Fathi H. A., Babu R. C. Rajeev K. S. Analava M. An Experimental Design Approach for Impurity Profiling of Valacyclovir-Related Products by RP-HPLC Sci Pharmaceutica 2014; 82: 617–630
10. Christina N. Anna O. Eleni E. David H. Ester H. Dimitra L. Analytical strategies for the determination of antiviral drugs in the aquatic environment . Trends in Environmental Analytical Chemistry ISSN 2214-1588

11. Mr. SOMAVEL. G DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ASSAY OF NEVIRAPINE AND ITS RELATED SUBSTANCES IN TABLET DOSAGE FORM.
12. Venkatnarayana M*, Siva Jyothi N Development of Validation and Stability Indicating Method of Anti-HIV Dolutegravir Drug and its Related Impurities by Using RP-HPLC Journal of Chromatography & Separation Techniques Vol.11 Iss.1 No:426
13. Sreenivasa Rao Chitturi a, Ch. Bharathi a, A.V. Raghava Reddya, K. Chandrasekhar Reddya, Hemant Kumar Sharma*, Vijay Kumar Handaa, Ramesh Dandalaa, V. Hima Bind Impurity profile study of lopinavir and validation of HPLC method for the determination of related substances in lopinavir drug substance Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1430–1440
14. Kondra Srinivasu^{1,2}, Bapuji A.T², D Gowri Shankar¹, Dr. Vijay Bharathi Dasari², Potturi Murali Krishnam Raju .Identification and Quantification Assessment of Impurity Profile Using Online Coupling of Single Quad Mass Detector with Photodiode Array Detector on Entecavir Drug Product. International Journal of Pharma Sciences and Scientific Research. ISSN 2471-6782 5:8, 91-108
15. María del Mar Contreras^{a,b,*}, Aránzazu Morales-Soto^{a,b}, Antonio Segura-Carretero^{a,b}, Javier Valverde^b. Potential of RP-UHPLC-DAD-MS for the qualitative and quantitative analysis of sofosbuvir in film coated tablets and profiling degradants. Journal of Pharmaceutical Analysis.
16. Vishal H. Wagh*, Sonia Singh, Rahul S. Buchade, Arshia Shariff, Sandeep D. Tandale, Pravin P. Chaur. Synthesis, Characterization and Development of Validated Analytical Methods for Process Related Impurity in Ritonavir Hydrochloride Bulk and Formulation. Journal of Pharmaceutical Science and research . Vol. 11(8), 2019, 2878-2885
17. Nourah Z. Hadir Mohamed M. Simultaneous determination of newly developed antiviral agents in pharmaceutical formulations by HPLC-DAD 1 (2017) 11:1
18. Girija B. Bhavar*¹, Sanjay S. Pekamwar², Kiran B. Aher¹, Sanjay R. C. Development and Validation of RP-HPLC Method for the Determination of Valacyclovir Hydrochloride and its Related Substances in Tablet Formulation. International Journal of Pharmaceutical Sciences Review and Research. 25(1), Mar – Apr 2014; Article No. 08, Pages: 53-58
19. Khalid A. Al-Amri, Kazi Mohsin *, Fars K. Development and validation of a UPLC method for quantification of antiviral agent, Acyclovir in lipid-based formulations. Arabian Journal of Chemistry ISSN 1878-5352.