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ANALYTICAL METHODS FOR THE RECENTLY APPROVED FDA NEW MOLECULAR ENTITIES – A REVIEW

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ABSTRACT:

Globally the R & D divisions of pharma industry are actively involved in the development of new therapeutic agents. These agents may be either new entities or partial structural modification of the existing one. The recent FDA statistics represent that the average number of drug filings are increasing every year in the thrust areas like anti-cancer agents, antibiotics, cardio-vascular drugs, respiratory drugs etc. Statistics signify that there is an increase in new drug approvals by 155% in anticancer drugs, 45% in antivirals, 14% in cardio-vascular drugs, and 700% in respiratory drugs and 100% in gastro-enterology drugs etc. in the past seven years. Very often there is a time lag from the date of introduction of a drug in to the market to the date of its inclusion in pharmacopeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions, standard and analytical procedures for these drugs may not be available in pharmacopeias. Therefore, it becomes necessary to develop and validate new analytical methods for such drugs. In this review, the methods for the analysis of such recently approved new molecular entities in various matrices are presented. This review helps in understanding the further need for the development of analytical methods for the estimation of such drugs.

Key words: FDA, analytical methods, validation.

INTRODUCTION:

Analytical method development and validation for the analysis therapeutic components and associated substances play an important role in the discovery, development and manufacture of pharmaceuticals and natural medicinal compounds. Pharmaceutical and herbal products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in a single dosage. The new chemical entities and combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products [1].

Analytical method development and validation though often considered routine, too little attention is paid to them with regards for their potential to contribute to overall developmental time and cost efficiency. These method- related activities are interrelated. They are iterative, particularly during early drug development phases. Parts of each process may occur concurrently or be refined at various phases of drug development. Changes encountered during drug development may require modifications to existing analytical methods. These modifications may require additional validation or transfer activities. In this paper, the overview the

different analytical methods is given according to the specific therapeutic categories of drugs.

1. ANALYSIS OF ANTI-CANCER AGENTS:

1.1. ALECTINIB

Alectinib is an oral drug that blocks the activity of anaplastic lymphoma kinase (and is used to treat non-small cell lung cancer (NSCLC). The chemical structure of Alectinib was represented in the figure 1 [2].

For the characterization of the PK of alectinib and its major human metabolite M4 in human plasma from clinical studies, two bioanalytical methods were developed, validated and cross-validated. Chugai used separate assays for alectinib and its metabolite M4 in a pivotal Phase I/II study, while Roche established a simultaneous assay for both analytes for another pivotal study and all other studies. The methods proved to be selective, specific, sufficiently sensitive, reproducible and robust for the analysis of large numbers of samples. However Cross-validation assessment revealed a bias between the two bioanalytical methods, which was confirmed with the clinical PK data between both pivotal studies using the different bioanalytical methods [3].

1.2. BELINOSTAT

Belinostat is a histone deacetylase inhibitor (HDAC) drug developed for the treatment of hematological malignancies and solid tumors. The chemical structure of Belinostat was represented in the figure 2 [4].

Two bioanalytical methods were developed for the analysis of Belinostat in human plasma. The first method was proposed by Jan H. Beumera et al. for the analysis of Belinostat and its five major metabolites in human plasma by using LC-MS/MS method. After protein precipitation, chromatographic separation was achieved with a Waters Acquity BEH C18 column and a linear gradient of 0.1% formic acid in acetonitrile and water. Detection with an ABI 4000Q mass spectrometer utilized both electrospray positive and negative mode ionization [5].

The second method was proposed by Ling-Zhi Wang et al. for determination of belinostat in plasma from liver cancer patients by using LC-MS/MS method. Chromatographic separation was achieved on a BDS Hypersil C18 column using gradient elution mode using 0.05% formic acid in water and 0.05% formic acid in acetonitrile as solvents A and B, respectively, 60/40. The run time was 6 min. The mass spectrometer was operated under a positive electrospray ionization condition and a multiple reaction monitoring mode. No significant matrix effect was identified. In analysis of patient samples, belinostat glucuronide was identified and baseline separated from belinostat [6].

1.3. CERITINIB

Ceritinib is a highly selective inhibitor of an important cancer target, anaplastic lymphoma kinase (ALK). It is used for the treatment of a specific type of lung cancer. The chemical structure of Ceritinib was represented in the figure 3 [7].

Olivier Heudi et al. developed Liquid chromatography tandem mass spectrometry method for the quantitative analysis of ceritinib in human plasma and its application to pharmacokinetic studies. The method consists of protein precipitation with acetonitrile, and salting-out assisted liquid liquid extraction (SALLE) using a saturated solution of sodium chloride prior to analysis by LC-MS/MS with electrospray ionization (ESI) technique in positive mode. Samples were eluted at 0.800 mL min−1 on Ascentis Express® C18 column with a mobile phase made of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). The method run time was 3.6 min and the low limit of quantification (LLOQ) was estimated at 1.00 ng mL−1 when using 0.100 mL of human plasma [8].

Rambabu Chintala et al. developed a simple stability indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method for the determination of ceritinib present in pharmaceutical dosage forms. The reported RP-HPLC method utilizes a BDS C18 Column in an isocratic separation mode. The mobile phase consists of 0.01 N potassium dihydrogen orthophosphate (KH_2PO_4) buffer and acetonitrile in the ratio 55:45 (%v/v). The elute was monitored by using the ultraviolet detector at 320 nm wavelength. The retention time of ceritinib was 2.539 minutes. The stability of the drug was examined under different stress conditions forcibly [9].

Naveen Kumar et al. a simple, stability indicating, elective, reproducible, economic, selective, RP- HPLC method for the estimation of Ceritinib both in bulk drug and in capsule dosage forms in the presence of degradation products. Reversed-phase chromatography was performed on a Kromosil C18 column with mobile phase Methanol: water (65:35 v/v) at a flow rate of 1.0 ml min-1. Detection was performed at 265nm and a sharp peak was obtained for Ceritinib at a retention time of 2.585 min. Stability indicating forced degradation established studies show results that there is no interference of any degraded products or external environmental conditions and it did not interfere with the detection of Ceritinib [10].

1.4. IBRUTINIB

Ibrutinib is an anticancer drug targeting B-cell malignancies. It is an orally-administered, selective and covalent inhibitor of the enzyme Bruton's tyrosine kinase (BTK).

The chemical structure of Ibrutinib was represented in the figure 4 [11].

A high performance liquid chromatography-tandem mass spectrometry (LC MS/MS) method was developed by Sridhar Veeraraghavan et al. for the simultaneous determination of lenalidomide, ibrutinib, and its active metabolite PCI45227 in rat plasma. Extraction of lenalidomide, ibrutinib, PCI45227 and tolbutamide (internal standard; IS) from 50 µl rat plasma was carried out by liquid-liquid extraction with ethyl acetate: dichloromethane (90:10) ratio. Chromatographic separation of analytes was performed on YMC pack ODS AM column under gradient conditions with acetonitrile: 0.1% formic acid buffer as the mobile phases at a flow rate of 1 ml/min. Precursor ion and product ion transition for analytes and IS were monitored on a triple quadrupole mass spectrometer, operated in the selective reaction monitoring with positive ionization mode [12].

Ronald de Vries et al. developed a quantitative LC-MS/MS methods for ibrutinib and metabolite dihydrodiol-ibrutinib in human plasma in the range 0.500–100 ng/ml. Selectivity of the assay toward isobaric metabolites and endogenous compounds was optimized and incurred sample reproducibility and stability were assessed. During analysis of plasma samples from a clinical study in hepatically impaired subjects, bile acids, more specifically taurocholic acid, showed interference with the internal standard of dihydrodiol-ibrutinib. This interference was significant in samples from hepatically impaired subjects, but did not impact results from all other clinical studies analyzed. The method was modified and revalidated, and an impact assessment was performed. The original method and the modified method were cross-validated.

Ayesha Tabassum et al. developed a simple, Precise, Accurate method for the estimation of Ibrutinib by RP-HPLC technique. Chromatographic conditions used are stationary phase Kromosil, Mobile phase 0.1% OPA: Acetonitrile in the ratio of 40:60and flow rate was maintained at 1ml/min, detection wave length was 296nm, column temperature was set to 30^{o}C and diluent was mobile phase Degradation studies of ibrutinib were done, in all conditions purity threshold was more than purity angle and within the acceptable range [13, 14].

1.5. LENVATINIB

Lenvatinib is an anti-cancer drug for the treatment of certain kinds of thyroid cancer, and potentially for other cancers as well. It acts as a multiple kinase inhibitor against the VEGFR1, VEGFR2 and VEGFR3 kinases. The chemical structure of Lenvatinib was represented in the figure 5 [15].

Uttam Prasad Panigrahy and Sunil Kumar Reddy developed a novel validated RP-HPLC-DAD method for the estimation of Lenvatinib Mesylate in bulk and pharmaceutical dosage form. Lenvatinib Mesylate were separated using Kromasil C18 column, Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of 0.01M Ammonium acetate and Methanol (30:70, v/v). The flow rate was set to 1ml/min with the responses measured at 309nm. The retention time of Lenvatinib Mesylate was found to be 3.733min [16].

1.6. OLAPARIB

Olaparib is a PARP inhibitor, inhibiting poly ADP ribose polymerase (PARP), an enzyme involved in DNA repair. It acts against cancers in people with hereditary BRCA1 or BRCA2 mutations, which include some ovarian, breast, and prostate cancers. The chemical structure of Olaparib was represented in the figure 6 [17].

Nijenhuis et al. developed a high-performance liquid chromatography–tandem mass spectrometry assay for quantifying olaparib in human plasma. Human plasma samples were collected in the clinic and stored at nominally −20 ◦C. Olaparib was isolated from plasma by liquid–liquid extraction, separated on a C18 column with gradient elution and analyzed with triple quadrupole mass spectrometry in positive ion mode. A deuterated isotope was used as internal standard for the quantification [18].

William D. Figg et al. developed a sensitive and Robust Ultra HPLC Assay with Tandem Mass Spectrometric Detection for the Quantitation of the Olaparib in Human Plasma for Pharmacokinetic Application. A liquid-liquid extraction was followed by chromatographic separation on a Waters UPLC® BEH C18 column and mass spectrometric detection. The mass transitions m/z 435.4→281.1 and m/z 443.2→281.1 were used for olaparib and the internal standard [2H8]-olaparib, respectively. Stability studies showed that olaparib is stable at room temperature for 24 h. in whole blood, at 4 °C for 24 h post-extraction, at −80 °C in plasma for at least 19 months, and through three freeze-thaw cycles [19].

1.7. TRABECTEDIN

Trabectedin is an anti-tumor drug used for the treatment of advanced soft tissue sarcoma. It is also undergoing clinical trials for the treatment of breast, prostate, and paediatric sarcomas. It has been shown that Trabectedin blocks DNA binding of the oncogenic transcription factor FUS-CHOP and reverses the transcriptional program in myxoid liposarcoma. The chemical structure of Trabectedin was represented in the figure 7 [20].

Massimo Zucchetti et al. developed a rapid, sensitive and specific HPLC–MS/MS for the quantification of trabectedin in human plasma after using deuterated trabectedin as Internal Standard (IS). Chromatographic separation was done on an Accucore XL C18 column using a Mobile Phase (MP) consisting of CH_3COONH_4 10 mM, pH 6.8 (MP A) and CH_3OH (MP B). The mass spectrometer worked with electrospray ionization in positive ion mode and Selected Reaction Monitoring (SRM), using target ions at [M−H2O+H]+ m/z 744.4 for trabectedin and [M−H2O+H]+ m/z 747.5 for the IS.

Massimo Zucchetti et al. also developed a HPLC–MS/MS method to measure trabectedin in tumors. They validated an LC–MS/MS assay determining the recovery, sensitivity, linearity, precision and accuracy in mouse tumor and liver samples. The limit of quantification was 0.10 ng/ml with a curve range of 0.10–3.00 ng/ml (accuracy 96.1–102.1 [21, 22].

1.8. IDELALISIB

Idelalisib is a drug used for the treatment of certain hematological malignancies. The substance acts as a phosphoinositide 3-kinase inhibitor; more specifically, it blocks P110δ, the delta isoform of the enzyme phosphoinositide 3-kinase. The chemical structure of Idelalisib was represented in figure 8 [23].

Sridhar Veeraraghavan et al. developed a method for Simultaneous quantification of idelalisib, fludarabine and lenalidomide in rat plasma by using high-performance liquid chromatography coupled with heated electrospray ionization tandem mass spectrometry Analytes were recovered by liquid–liquid extraction and separated on a reverse phase C18column using methanol: 0.1%formic acid buffer (70:30) as mobile phase at a flow rate of 1 mL/min in isocratic mode. Selective reaction monitoring was performed using the transitions, i.e. m/z 416.25/176.48, 286.11/154.10,260.15/149.15, and 271.14/155.06 to quantify idelalisib, fludarabine and lenalidomide and tolbutamide, respectively. The method was validated over the concentration range of 1.15–576.84 ng/mL for idelalisib, 0.95–476.25 ng/mL for fludarabine and 0.97–486.19 ng/mL for lenalidomide [24].

2. ANALYSIS OF ANTI-VIRAL AGENTS:

2.1. PERAMIVIR

Peramivir is an antiviral drug used for the treatment of influenza. Peramivir is a neuraminidase inhibitor, acting as a transition-state analogue inhibitor of influenza neuraminidase and thereby preventing new viruses from emerging from infected cells. The chemical structure of Peramivir was represented in the figure 9 [25].

Lindegardh et al. developed high-throughput zwitter-ionic hydrophilic interaction liquid chromatography solid-phase extraction–liquid chromatography–tandem mass spectrometry method for determination of the anti-influenza drug peramivir in plasma. The ZIC-HILIC SPE efficiently removed sources of interference present in the supernatant after protein precipitation of plasma proteins. The main advantage of the ZIC-HILIC SPE sample preparation step was that it allowed load and elution conditions to be optimized to extract only peramivir and minimize co-extraction of lipophilic phospholipids [26].

Ying Li et al. developed a method for quantification of peramivir in dog plasma by liquid chromatography /tandem mass spectrometry employing precolumn derivatization. The sample preparation consisted of a protein precipitation extraction followed by derivatization with 10 M hydrochloric acid–methanol (10:90, v/v) and determined by liquid chromatography coupled with tandem mass spectrometry. The selected reaction monitoring mode of the positive ion was performed and the precursor to the product ion transitions of m/z 343 → 284 and m/z 299 → 152 were used to measure the derivative of peramivir and Ro 64-0802 (internal standard, an active metabolite of oseltamivir). The chromatographic separation was achieved using a ZORBAX RX-C8 analytical column with an isocratic mobile phase composed of acetonitrile–water–formic acid (30:70:0.1, v/v/v, 0.2 mL/min) [27].

Yu-Guan Wen et al. developed a rapid LC–MS/MS quantification of peramivir using a simple and inexpensive sample precipitation: application to PK. They reported a robust and sensitive method utilizing simple precipitation extraction with LC–MS/MS for the high-throughput quantification. Addition of 0.06 M of ammonium formate and 0.1% formic acid in mobile phase could help reduce the matrix effect. This method uses 100 µl of plasma and covers a linear concentration range from 5 to 10,000 ng/ml [28].

2.2. DACTLASAVIR

Daclatasvir is an anti-viral drug for the treatment of hepatitis C (HCV). It inhibits the HCV nonstructural protein NS5A.Recent research suggests that it targets two steps of the viral replication process, enabling rapid decline of HCV RNA. The chemical structure of Daclatasvir was represented in the figure 10 [29].

Mamdouh R. Rezk et al. developed a sensitive and rapid UPLC-MS/MS method for quantitative determination of daclatasvir in human plasma: Application to a bioequivalence study. The Xevo TQD LC-MS/MS was operated under the multiple reaction monitoring mode using electrospray ionization. The prepared samples were chromatographed on Acquity UPLC HSS C18 column by pumping 10 mM ammonium formate (pH 3.5) and acetonitrile in an

isocratic mode at a flow rate of 0.30 ml/min. A very short run time of 1.2 minutes made it possible to analyze more than 500 human plasma samples per day.

Ashok Chakravarthy and Sailaja developed a stability indicating reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of Daclatasvir in tablet dosage forms for Assay and Dissolution methods. The developed method for Assay and Dissolution testing of Daclatasvir in tablets was carried out on Zorbax Eclipse plus C-18 column using Water and Methanol (20:80) by isocratic run. Flow rate was 1.5 mL min-1 with a column temperature of 25°C and detection wavelength was carried out at 315 nm. Total run time for the chromatographic analysis was 2 minutes for both assay and dissolution tests. The forced degradation studies were performed for assay content on Daclatasvir tablets under acidic, basic, oxidation, thermal, humidity and photolytic conditions. No degradation products were observed from the forced degradation studies [30, 31].

2.3. LEDIPASIVIR AND SOFOSBUVIR

Ledipasvir is a drug for the treatment of hepatitis C. Ledipasvir is an inhibitor of the hepatitis C virus NS5A protein. Sofosbuvir is a nucleotide analog used in combination with other drugs for the treatment of hepatitis C virus (HCV) infection. Sofosbuvir inhibits the RNA polymerase that the hepatitis C virus uses to replicate its RNA. The ledipasvir/sofosbuvir combination is a direct-acting antiviral agent that interferes with HCV replication and can be used to treat patients with genotypes 1a or 1b without PEG-interferon or ribavirin.The chemical structures of Sofosbuvir and Ledipasvir were represented in the figures 11 and 12 respectively [32, 33].

Zhenzhen Pan et al. developed a method for the simultaneous determination of ledipasvir, sofosbuvir and its metabolite in rat plasma by UPLC–MS/MS and its application to a pharmacokinetic study. The analytes and the internal standard (diazepam) were separated on an Acquity UPLC BEH C18 chromatography column using gradient elution with a mobile phase of acetonitrile and 0.1% formic acid in water at a flow rate of 0.4 mL/min [34].

The detection was performed on a triple quadrupole tandem mass spectrometer by multiple reaction monitoring(MRM) mode to monitor the precursor-to-product ion transitions of m/z 889.8 → 130.1 for ledipasvir, m/z 530.3 → 243.1 for sofosbuvir, m/z 261.5 → 113.1 for GS-331007 and m/z 285.2 → 193.1 for diazepam(IS) using a positive electrospray ionization interface. Total time for each chromatography was 3.0 min.

3. ANALYSIS OF CARDIO-VASCULAR RELATED DRUGS

3.1. EDOXABAN

Edoxaban is an oral anticoagulant drug which acts as a direct factor Xa inhibitor. It is used for prevention of venous thromboembolisms (VTE) following lower-limb orthopedic surgery. The chemical structure of Edoxaban was represented in the figure 13 [35].

Tracey Gous et al. developed a Turbulent Flow Liquid Chromatography with High-Resolution Mass Spectrometry for the measurement of the Direct Oral Anticoagulants Apixaban, Dabigatran, Edoxaban, and Rivaroxaban in Human Plasma. Samples, calibrators, and internal quality controls (100 mL) were mixed with internal standard solution (50 mg/L both dabigatran-$^{13}C_6$ and rivaroxaban-$^{13}C_6$ in acetonitrile) and, after centrifugation (16,400g, 4 minutes), supernatant (100 mL) was injected onto a Cyclone-C18-P-XL TurboFlow column. Analytes were focused onto an Accucore PhenylHexyl analytical column and eluted using a methanol + acetonitrile (1 + 1): aqueous ammonium acetate (10 mmol/L) gradient. Data were acquired using high-resolution mass spectrometry in full-scan mode (100–2000 m/z) with data-dependent fragmentation to confirm peak identity [36].

Pasam Satyanarayana Reddy et al. developed a Stability Indicating High-Performance Liquid Chromatography for the determination of Edoxaban in bulk and in tablet dosage form. Edoxaban was separated on Hypersil BDS C18 column using 0.1M K_2HPO_4: Methanol (65:35, v/v) as an isocratic mobile phase at a flow rate of 1.0 ml/min. Detection was performed using photodiode array detector set at 245 nm. Edoxaban was eluted at 3.785 min with a total run time of 6 min. The stability indicating nature of the method was established by subjecting the edoxaban to stress conditions such as acid and base hydrolyses, oxidative, photo- and thermal degradations. The degraded products formed in all stress conditions were resolved successfully from the edoxaban [37].

3.2. VORAPAXAR

Vorapaxar is a thrombin receptor (protease-activated receptor, PAR-1) antagonist based on the natural product himbacine. The chemical structure of Vorapaxar was represented in the figure 14 [38].

Cristina I. Tama et al developed an Ultra Performance Liquid Chromatography TM–tandem mass spectrometry for routine bioanalytical analysis of Vorapaxar. To permit accurate determinations of circulating SCH 530348 in plasma following dosing, a method for measuring SCH 530348 concentrations in human plasma was validated using liquid chromatography–tandem mass spectrometry (LC–MS/MS).

The method utilized semi-automated 96-well protein precipitation with gradient chromatography using an ACQUITYTM UPLC BEH C18 column. The retention time of SCH 530348 was approximately 1.5 min [39].

3.3. IVABRADINE

Ivabradine is a medication used for the symptomatic management of stable heart related chest pain and heart failure not fully managed by beta blockers. It acts by reducing the heart rate via specific inhibition of the funny channel, a mechanism different from that of beta blockers and calcium channel blockers, two commonly prescribed antianginal drugs. The chemical structure of Ivabradine was represented in the figure 15 [40].

Shweta Maheshwari et al. a stability indicating RP-HPLC Method and Spectrophotometric Method for the quantitative determination of Ivabradine in solid dosage form. Separation and detection of Ivabradine HCl by HPLC was achieved by Inertsil ODS-3V column and U.V. detector at 286 nm respectively. In HPLC method the retention time was about 7 minutes.

Selva Kumar et al. developed a stability indicating rapid HPLC method for estimation of Ivabradine hydrochloride in solid oral dosage form. The chromatographic separation was obtained using a mobile phase composition at a ratio of 50:50 (v/v) of 10 mM ammonium acetate buffer pH 6.0 and methanol on Phenomenex Kinetex C18 column, ambient temperature with UV detection at 285 nm at a flow rate of 1.0 ml/minute. The retention was at 3.1 mins. The peak purity plots show that the Ivabradine hydrochloride peak is homogeneous and that there are no co eluting peaks indicating that the method is stability indicating and specific [41, 42].

Gandhimathi et al. developed high performance thin layer chromatography and high performance liquid chromatographic techniques for Ivabradine hydrochloride in bulk and tablet dosage form. The HPTLC separation was carried out on aluminium sheet of pre-coated silica gel 60 F254 using Methanol: Diethyl ether: Cyclohexane: 10% triethyl amine in the ratio of (8:0.5:0.5:1%v/v/v/v) as mobile phase followed by analysis at 287nm. The RP-HPLC separation of ivabradine was performed on a LichroCart®250-4 column with a mobile phase consist of 45:55v/v of 5% Triethyl amine: methanol (buffer pH adjusted to 7 with ortho phosphoric acid) with the flow rate of 1ml/min and the detection at 286nm.The retention time for Ivaradine HCl was found to be 6.5min [43].

4. ANALYSIS OF ANTIBIOTICS

4.1. DALBAVANCIN

Dalbavancin is a novel second-generation lipoglycopeptide antibiotic. Similar to other glycopeptides, dalbavancin exerts its bactericidal effect by disrupting cell wall biosynthesis. It binds to the D-alanyl-D-alanyl residue on growing peptidoglycan chains and prevents transpeptidation from occurring, preventing peptidoglycan elongation and cell wall formation. The chemical structure of Dalbavancin was represented in figure 16 [44].

Marco Cavaleri et al. reported the pharmacokinetics and excretion of dalbavancin in the rat. A 20 mg/kg intravenous dose of dalbavancin or [3H] dalbavancin was administered to rats in three studies. Concentrations of dalbavancin or drug-derived radioactivity were assessed in blood, plasma, tissues, bile, urine and faeces by HPLC-MS/MS, scintillation counting or microbiological methods. These data were useful in designing and interpreting animal infection model studies used to select the dose for human studies [45].

4.2. FINAFLOXACIN

Finafloxacin is a fluoroquinolone antibiotic. It is used to treat acute otitis externa (swimmer's ear) caused by the bacteria Pseudomonas aeruginosa and Staphylococcus aureus. The chemical structure of Finafloxacin was represented in figure 17 [46].

WANG Dong et al. developed a HPLC method for the determination of R,R isomer in finafloxacin hydrochloride. The separation was performed on a Chiralpak AY-H column with n-heptane- isopropanol-diethylamine (70:25:0.1) as mobile phase. The flow rate was 1.0 mL/min with Injection volume of l0µL, the column temperature was 35 ^{0}C and the detection wavelength was set as 295 nm. Finafloxacin hydrochloride and R,R Isomer had good linearity in the ranges of 101.2 - 813.1 ng/mL and 115.6 - 817.8 ng/mL. The method is convenient and accurate for split check of R.R-finafloxacin hydrochloride Isomer which can be used in quantity control for finofloxacin hydrochloride [47].

4.3. TEDIZOLID PHOSPHATE

Tedizolid is an oxazolidinone-class antibiotic. It is used for the treatment of acute bacterial skin and skin structure infections. Tedizolid phosphate is a prodrug activated by plasma or intestinal phosphatases to tedizolid following administration of the drug either orally or intravenously. Once activated, tedizolid exerts its bacteriostatic microbial activity through inhibition of protein synthesis by binding to the 50S ribosomal subunit of the bacteria. The chemical structure of Tedizolid was represented in figure 18 [48].

Nicolau et al. developed a High Performance Liquid Chromatography method for the determination of Tedizolid in human plasma, human serum, saline and mouse plasma. An ultra violet detector set at 251nm was used with a reverse phase column. The mobile

phase consisted of sodium acetate, deionized water and acetonitrile at a flow rate of 1.0 ml/min. 4-nitroaniline was used as the internal standard. This method was successfully used to assay samples previously analyzed by liquid chromatography with tandem mass spectrometric detection [49].

4.4. ISAVUCONAZOLE

Isavuconazole is a triazole antifungal drug. Its prodrug, isavuconazonium sulfate. Isavuconazole works by inhibiting lanosterol 14 alpha-demethylase, the enzyme responsible for converting lanosterol to ergosterol by demethylation. The resulting depletion of ergosterol and buildup of lanosterol compromise the structure of the fungal cell membrane. Mammalian cells are resistant to demethylation inhibition by azoles, making the drug effects specific to fungi. The chemical structure of Isavuconazole was represented in figure 19 [50].

Verweij-van Wissen et al. developed a bioanalytical method for the simultaneous determination of the azoles voriconazole, posaconazole, isavuconazole, itraconazole and its metabolite hydroxy-itraconazole in human plasma by reversed phase ultra-performance liquid chromatography with ultraviolet detection. The method involved a simple liquid–liquid extraction followed by ultra-performance liquid chromatography with an Acquity UPLC BEH Phenyl column and ultraviolet detection set at a wavelength of 260 nm. This method proved to be simple, accurate, precise and fast and is currently in use in our laboratory for the quantitative analysis of these azoles for Therapeutic Drug Monitoring and pharmacokinetic research [51].

Spandana et al. developed a RP-HPLC Method for the Estimation of Isavuconazole in Bulk and Formulation. A simple, rapid, precise and accurate RP-HPLC method was developed and validated for the estimation of Isavuconazole. An Inertsil(R) column kept at ambient temperature, using a mobile phase consisting of 0.1M Ammonium acetate: methanol (60:40) at a flow rate of 1.2 ml/min and UV detection at 251nm. Due to its simplicity, rapidness and high precision, the method was successfully applied to the estimation of Isavuconazole [52].

5. ANALYSIS OF DRUGS USED IN THE TREATMENT OF DIABETES

5.1. DAPAGLIFLOZIN PROPANEDIOL

Dapagliflozin is a drug of the gliflozin class, used to treat type 2 diabetes. It inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2) which are responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter mechanism causes blood glucose to be eliminated through the urine. The chemical structure of Dapagliflozin was represented in figure 20 [53].

Manasa Sanagapati et al. developed a stability-Indicating RP-HPLC method and UV-Spectroscopy for determination of Dapagliflozin. The proposed method utilizes BDS column (maintained at ambient temperature), gradient run (using mixture of acetonitrile and ortho phosphoric acid as mobile phase), effluent flow rate (1ml/min) and detection at 245nm using PDA detector. The stability of the drug was determined by studying the degradation of the drug under acidic, alkaline, peroxide, neutral, heat and UV conditions. RP-HPLC method [54, 55].

Mohammad Yunoos and Gowri Sankar developed a stability indicating high-performance liquid chromatographic method for simultaneous determination of Metformin HCl and Dapagliflozin in bulk drug and tablet dosage form. Chromatography was carried out on hypersil BDS C18 column containing mobile phase of buffer (0.1% orthophosphoric acid) adjusted to pH 6.8 with triethylamine:acetonitrile in the ratio of 50:50%/v/v at a flow rate of 1 ml/minutes. The analyte was monitored using photodiode array detector at 240 nm. The retention time was found to be 2.791 minutes and 3.789 minutes for MET hydrochloride and DAP respectively [56].

5.2. EMPAGLIFLOZIN

Empagliflozin is a drug of the gliflozin class, approved for the treatment of type 2 diabetes in adults. It is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), which is found almost exclusively in the proximal tubules of nephronic components in the kidneys. The chemical structure of Empagliflozin was represented in figure 21 [57].

Bassam M. Ayoub developed an UPLC Simultaneous Determination of Empagliflozin, Linagliptin and Metformin. The first UPLC method was developed for simultaneous determination of empagliflozin, linagliptin and metformin hydrochloride in their different combinations as pharmaceutical dosage forms. Chromatographic separation was achieved on a Symmetry® AcclaimTM RSLC 120 C18 column applying an isocratic elution based on potassium dihydrogen phosphate buffer pH (4) - methanol (50:50, v/v) as a mobile phase. All the variables were studied to optimize the chromatographic conditions [58].

Padmaja and Veerabhadram developed a method for Simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy. Simple, precise and economical UV Spectrophotometric methods have been developed for the simultaneous estimation of Empagliflozin and Linagliptin in bulk and pharmaceutical dosage forms. The simultaneous equation (Vierodt's Method), which is based on

measurement of absorption at 233nm and 277nm i.e. λmax of Empagliflozin and Linagliptin respectively [59].

Padmaja and Veerabhadram also developed a RP-HPLC method for the estimation of Empagliflozin in API. An accurate, precise and rapid RP-HPLC method was developed and subsequently validated for the determination of Empagliflozin in API. Better separation of the drug was achieved on Intersil column with the mobile phase consisted of mixture of 0.01 M acetate buffer, methanol in ratio of (30:70v/v) at flow rate of 2ml/min, with detection at 260nm using PDA detector. The retention time was found to be 1.223min.

Shyamala et al. developed a stability-indicating RP-HPLC method for determination of Empagliflozin. The present work describes the development and following validation of a stability indicating reverse phase HPLC (RP-HPLC) method for the analysis of Empagliflozin in its API. The proposed method utilizes Hypersil BDS column Mobile phase 0.1% OPA: Acetonitrile in the ratio of 70:30 and flow rate was maintained at 1ml/min, detection wave length was 233nm, and column temperature was set to 30°C. The stability of the drug was determined by studying the degradation of the drug under acidic, alkaline, peroxide, neutral, heat and UV conditions [60, 61].

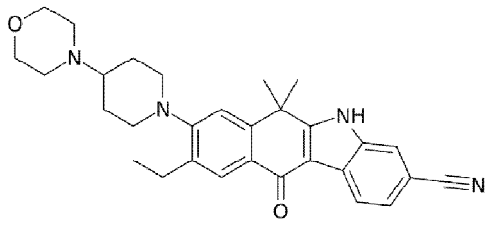

Figure 1. Chemical Structure of Alectinib

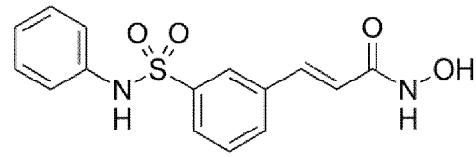

Figure 2. Chemical Structure of Belinostat

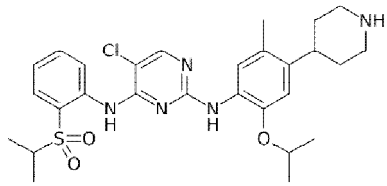

Figure 3. Chemical Structure of Ceritinib

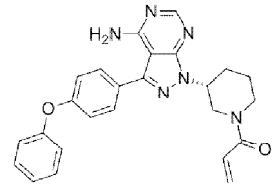

Figure 4. Chemical Structure of Ibrutinib

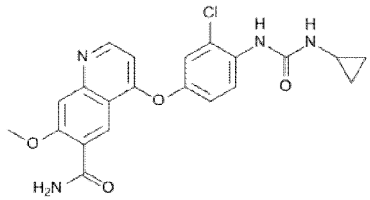

Figure 5. Chemical Structure of Lenvatinib

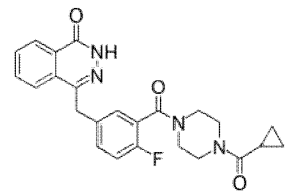

Figure 6. Chemical Structure of Olaparib

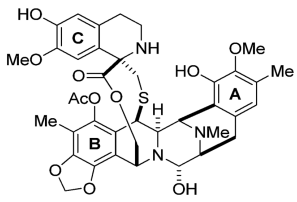

Figure 7. Chemical Structure of Trabectedin

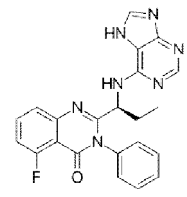

Figure 8. Chemical Structure of Idelalisib

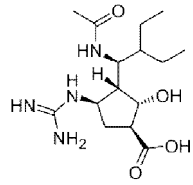

Figure 9. Chemical Structure of Peramivir

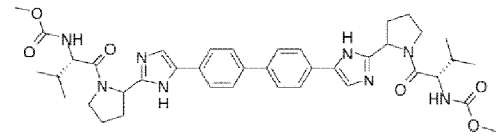

Figure 10. Chemical Structure of Dactlasavir

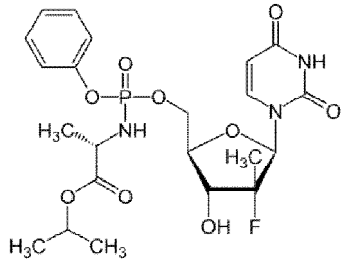

Figure 11. Chemical Structure of Sofosbuvir

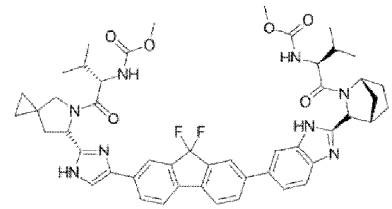

Figure 12. Chemical Structure of Ledipasivir

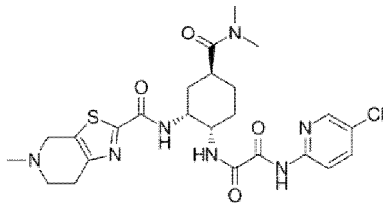

Figure 13. Chemical Structure of Edoxaban

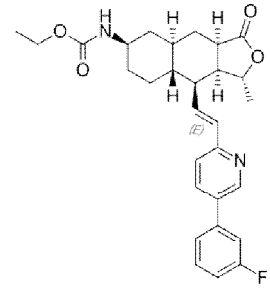

Figure 14. Chemical Structure of Vorapraxar

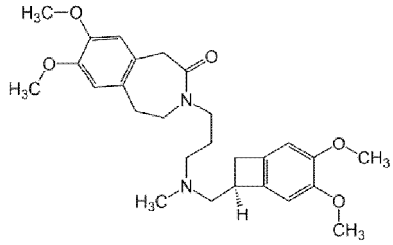

Figure 15. Chemical Structure of Ivabradine

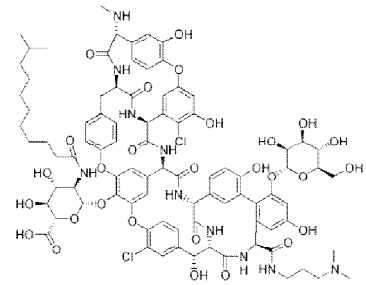

Figure 16. Chemical Structure of Dalbavancin

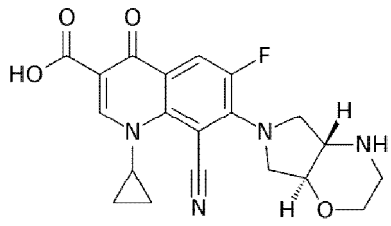

Figure 17. Chemical Structure of Finafloxacin

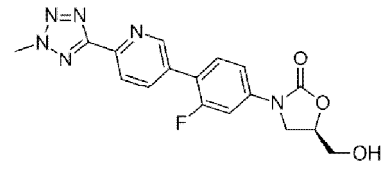

Figure 18. Chemical Structure of Tedizolid

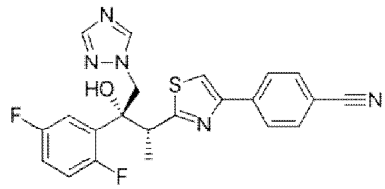

Figure 19. Chemical Structure of Isavuconazole

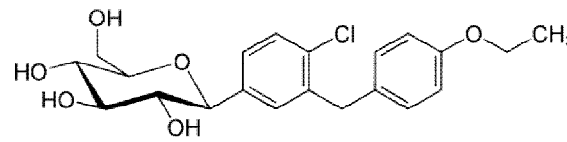

Figure 20. Chemical Structure of Dapagliflozin

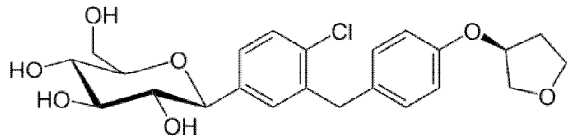

Figure 21. Chemical Structure of Empagliflozin

6. CONCLUSIONS:

The new chemical entities and combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. Analytical method development and validation though often considered routine, too little attention is paid to them with regards for their potential to contribute to overall developmental time and cost efficiency. These method- related activities are interrelated. They are iterative, particularly during early drug development phases. Parts of each process may occur concurrently or be refined at various phases of drug development. Changes encountered during drug development may require modifications to existing analytical methods. These modifications may require additional validation or transfer activities.

Over the past three years around 60 new molecular entities were approved by United States Food and Drug Administration. Among them, drugs belonging to various therapeutic categories like anti-cancer, drugs related to cardio-vascular diseases, antiviral, antibiotic, drugs used in the treatment of diabetes were represented in this paper. Analytical methods for the determination of such drugs in pharmaceutical formulations, biological samples, and environmental samples have been reported in the literature. The developed methods supported in understanding the pharmacokinetics, pharmacodynamics, therapeutic drug monitoring, drug interactions with the organism, developing pharmaceutical formulations, determining the toxicity of these compounds. Nevertheless there is a scope for the development of analytical methods which are time and cost effective, environmental friendly.

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