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CHROMATOGRAPHIC METHODS FOR THE SELECTED ANTIVIRAL DRUGS: A REVIEW

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ABSTRACT

The detection and measurement of biomolecules and metabolites in animal and human tissues utilizing bimolecular approaches requires the adoption of a procedure. The bioanalytical approach is an accurate way to count the quantity of substances and metabolites that are present in a biological system. The development of new techniques, the verification of already established protocols, and the examination of specimens are among the most important aspects of bioanalysis. First and foremost, a molecule may be measured using many ways, and it can be recognized using a variety of analytical approaches. Extraction methods such as protein precipitation in complicated plasma and biological samples, liquid extraction, and solid-phase extraction are some of the ways that drugs may be examined for quality control purposes. The linearity, specificity, sensitivity, reproducibility, accuracy, selectivity, and recovery of an assay are the validation factors that are taken into consideration. In order to contribute to the process of ensuring the high quality of pharmaceuticals, we propose the creation and validation of bioanalytical systems.

Keywords: LC-MS/MS, Bioanalytical technique, Extraction techniques, Method validation

INTRODUCTION

Analytes in biological samples, such as plasma, blood, saliva, serum, urine, skin, feces, hair, and organ tissue, may be identified and quantified by the process of bioanalysis. Bioanalysis uncovers the presence of drug and metabolite components, in addition to the presence of protein and peptide molecules. Bioanalysis is an essential part of the process of developing new drugs, conducting forensic investigations, monitoring doping, and identifying sickness biomarkers. The sample matrix adds a layer of complexity to the bioanalysis **[1]**. Methods from bioanalysis are used in the creation of new drugs. Validation of bioanalytical techniques is required in order to quantitatively analyze analytes in biological matrices. These days, sample solution preparation for hyphenated devices is necessary before conducting bioanalysis. For the purposes of drug discovery and development, pharmaceutical research companies need comprehensive bioanalytical methods **[2-11]**. In order to get accurate results from analytical equipment used on complex matrices like blood, plasma, and urine, significant sample preparation is required. Sample preparation and hyphenated analytical processes are required in contemporary bioanalysis in order to successfully recover analytes. Utilized for a very long time in medical LCMSMS for bioanalysis.

CABOTEGRAVIR

Cabotegravir is an antiretroviral medicine that is utilizing for management of AIDS/ HIV. It is also offered as the trade name Vocabria, amongst other brand names. Tablets, an intramuscular injection, and a combined injectable formulation with rilpivirine sold under the trade name Cabenuva are the various delivery methods that are available for this medication. Integrase strand transfer is something that cabotegravir can stop from happening. This implies that it inhibits the enzyme integrase that is produced by HIV, which stops the virus's genome from being incorporated into the DNA of human cells. Because the virus cannot reproduce without this stage being completed, it is more difficult for it to propagate farther **[12]**.

Adults who are infected with human immunodeficiency virus type 1 (HIV-1) and are seeking therapy may benefit from taking cabotegravir in conjunction with rilpivirine.. Before beginning the injectable therapy, the tablets are taken to determine whether or not the patient can tolerate the medicine being administered. These two medications are the first antiretroviral treatments that are available in a formulation that is long-acting and injectable **[13]**.

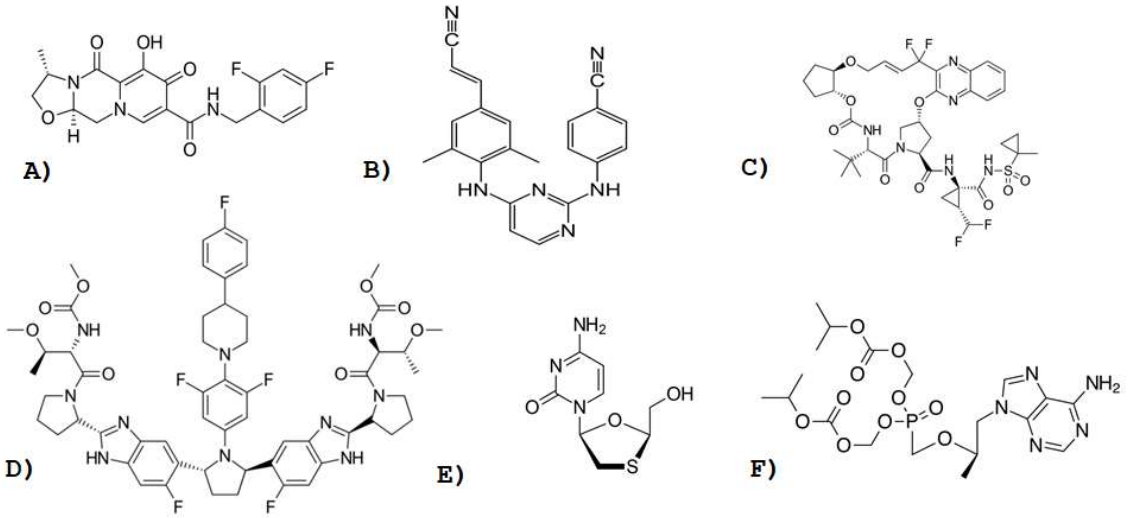

Figure 1: Structure of A) Cabotegravir, B) Rilpivirine C) Glecaprevir D) pibrentasvir E) Lamivudine and F) Tenofovir disoproxil

RILPIVIRINE

Rilpivirine is a medicine produced by Tibotec that is used for the treatment of AIDS/ HIV. This medication is marketed under the trade names Edurant and Rekambys. It is a II-generations non-nucleosides reverse transcriptase inhibitor (NNRTI), which means that it has a greater efficacy, a longer half-life, and a lower side-effect profile in comparison to previous NNRTIs such as efavirenz. The IUPAC of Rilpivirine is 4-{[4- ({4- [(E)- 2-cyanovinyl] -2, 6-dimethylphenyl} amino) pyrimidin-2-yl] amino}benzonitrile with an empirical formula of $C_{22}H_{18}N_6$ with a molecular weight of 366.428 g.mol^{-1} **[14]**.

The DNA of the virus becomes incorporated into the chromosomal DNA of the host, which makes it possible for the host's cellular activities, including as transcription and translation, to multiply the virus. RTIs inhibit the enzymatic process of reverse transcriptase, which stops the virus from replicating itself and stops the completion of the creation of double-stranded viral DNA. Other kinds of viruses go through a procedure that is analogous to this one. For instance, the hepatitis B virus stores its genetic information in the form of DNA and replicates with the help of an RNA-dependent DNA polymerase. Some of the same chemical compounds that are used in the treatment of RTIs are also capable of inhibiting HBV replication; when utilized in this manner, these compounds are referred to as polymerase inhibitors **[15]**.

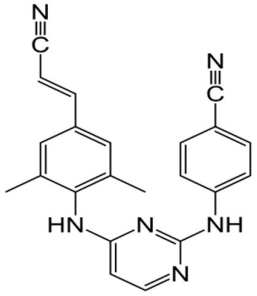

Figure 2: Structure of B) Rilpivirine

GLECAPREVIR

It is inhibits the HCV NS34A proteases. This protease is viral enzymes that are required for the proteolytic cleavage of the HCV encoded polyprotein into mature versions of the NS3, NS4A, NS4B, and NS5A and NS5B proteins Label. Glecaprevir prevents this cleavage from occurring. For viral replication to take place, certain proteins with several functions, including NS3, are required. The serine protease activity is encoded in the N-terminal region of the NS3 protein, whereas the C-terminal portion of the NS3 protein encodes a DExH/D-box RNA helicase. Both halves of the NS3 protein are required for the virus to replicate. During the process of replication of viral genomic RNA, this RNA helicase uses the hydrolysis of NTP as a source of energy in order to unwind double-stranded RNA in a 3' to 5' direction. NS4A is a cofactor for NS3 that affects where NS3 is placed and how it operates enzymatically. NS3 is responsible for the replication of the NS3 virus. It does this by directing the actions of NS3. Glecaprevir is able to accomplish this goal by inhibiting the activity of the NS3/4A protease, which is the enzyme that is accountable for cleaving downstream junctions of HCV polypeptide and the proteolytic processing of mature structural proteins. This creates a disturbance in the processes of the viral life cycle that take place inside of the cells **[16-18]**.

PIBRENTASVIR

Adult patients with chronic hepatitis C virus (HCV) genotype 1, 2, 3, 4, 5 or 6 infection without cirrhosis or with compensated cirrhosis (Child-Pugh A) are candidates for therapy with this medication. MAVYRET is also permitted for treating of adults with HCV genotypeI infections who have been treated in the past with a regimen comprising either an HCV NS5A inhibitor or an NS3/4A protease inhibitor (PI), but not both. These patients have had prior therapy with a regimen containing either an HCV NS5A inhibitor or an NS3/4A PI. NS5A is a phosphoprotein that is involved in the replication, assembly, and maturations of communicable viral proteins. It is required to all three of these processes. When it comes to ensuring that NS5A interacts with viral capsid proteins, also known as the core proteins, the basally phosphorylation forms of NS5A was essential. This form is maintained by the C-terminals serine clusters. Pibrentasvir is able to prevent assembly of protein and the formation of mature HCVs particle because it is able to disrupt this interaction. In addition, NS5A participates in the formation of the HCV replicase complex by interacting with both viral and cellular proteins. It also plays a role in the synthesis of HCV's RNA **[19]**.

LAMIVUDINE

It is a reverse transcriptase blocker that is also an analogue of zalcitabin; in this compound, the 3' carbon of the pentose ring is replaced by a sulfur atom. Both human immune deficiency viruses type I (HIV-I) and hepatitisB viruses (HBVs) may be cured with this medication. Lamivudine is a nucleosides reverse transcriptase blocker (NRTI) that may impair the production of viral DNA and is effective HIV-1 and HBV. It may produce active metabolite substances, which then compete with each other to be incorporated into viral DNA, when it is phosphorylated. Lamivudine metabolites, when incorporated into DNA, both function as a chain terminator in DNA synthesis and block the action of HIV enzyme reverse transcriptase via the process known as competitive inhibition. Incorporating nucleoside analogues into a molecule prevents the creation of a 5' to 3' phosphodiester linkage, which is necessary for the elongation of a DNA strand. This is caused by the absence of a 3'-OH groups in a molecule **[20]**.

TENOFOVIR DISOPROXIL

Viread, also known as tenofovir disoproxil fumarate, was a kind of antiretroviral medication that is categorized as nRTI(nucleotide analogue reverse transcriptase inhibitor). This type of antiretroviral medication is produced by Gilead Sciences and sold under the brand name Viread. Both the treating HBV and the management of HIV infections need the use of this medication in conjunction with a number of other medications. Tenofovir disoproxil was granted approval for use for the first time in the year 2001. This medication inhibits enzymes that are essential for viral duplication in HIV1 and HBV infections, therefore preventing the elongation of viral DNA chains **[21]**.

Tenofovir diphosphate also causes viral DNA chain termination after it has been integrated into DNA. The IUPAC name of tenofovir disoproxil is Bis{[(isopropoxy carbonyl) oxy] methyl }({[(2R)-1-(6-amino-9H-purin -9-yl)-2-propanyl oxy} methyl) phosphonate with an empirical formula of $C_9H_{14}N_5O_4P$ with a molecular weight of 229.25 $g.mol^{-1}$.

The phase of the analysis known as sample processing, is often the most time-consuming and difficult aspect of the process. This is because it is necessary to remove the required analyst from the matrix, which may be a complex process. In addition, each matrix has its own particular set of challenges. Urine has large concentrations of salt; for instance, plasma contains an abundance of phospholipids. The components that make up whole blood include red blood cells, lysed blood cells, and so on. There are often differences in the characteristics of each analyte and matrix,

which determine the kind of extraction technique that must be used **[22-24]**.

The aims of sample preparation are as follows: 1. lessen the effect of the matrix, 2. Take a sample off for use in determining the sample's variability. 3. Decrease the amount of variation in the tests, 4. an increased level of sensitivity, 5. The samples should be in better condition, 6. Being aware of the difficulties involved in the creation of new methods **[25]**, 7. A look at the matrix The total number of samples taken, 8. a swift course of action, 9. The number of analyses used in the quantification, 10. conduct an analysis of the pharmacological profile, 11. The proportion of blood to plasma in the body.

The ratio of a drug's concentrations in a blood to its concentration in plasma is the percentage of drug's concentrations in the blood to corresponding concentration in the plasma in whole blood (also known as CB/CP) **[26]**.

SAMPLE EXTRACTION TECHNIQUES

Solid phase extraction (SPE)

It is predicated on either a phenomena of adsorption using the particulate materials(an adsorbents) to a particular compound is partitioning below a predetermined set of circumstances or the selected analyte adsorption by the solid adsorbents. Function of SPE is determined by an adsorption method that is used. Using desired solvent, a target analytes may be extracted or/and isolated from the sample **[27]**.

Liquid Liquid Extraction (LLE)

It is contingent upon a selective extractions of a materials from a liquid utilizing immiscible organic solvent of analytes that is present in it. A wide variety of hydrocarbons, as well as ethylene dichloride and diethyl acetate, are used as solvents. In order to be successful, liquid-liquid extraction has to produce differential solubility and then separate two fluids that cannot mix. The two stages have to be integrated into one another. In most cases, there will be two stages: the first will be water-based, and the second will include live creatures. In the event that it is necessary to do so, the extraction phase may be eliminated from the matrix.

Protein precipitation

If we take a biological matrix as an example, the solubility of an analyte like glucose is going to be determined by the main solvent, which may be something like plasma. Methanol and acetyl chloride (ACN) are examples of solvents. The proteins are fully denatured as a result. It is possible to precipitate the vast majority of the protein using urea **[28-30]**. In order to achieve the desired pH level, the sample also receives organic solvents. Because it is hydrophobic, it results in the protein being denatured. Recent advancements have been made in sampling and tuning technologies.

Table 1: Reported methods for the combination of Cabotegravir and Rilpivirine

Method	Column	Mobile phase	Detection	Flow rate	Authors
LC	SymmetryC18(4.6×150mm)	ACN, and 0.1% HCOOH in a 20:80 v/v	231 nm	1 mL/min	Vejendla, A *et al*., [31]
HPLC-MS	Scientific Apex Inertsil ODS column -3 (4.6mm×25 mm, 5µ)	ACN and 0.1%(v/v) trifluoroacetic acid in H_2O (8.1:1.9v/v)	(m/z) of RPV at 367.4 and CAB at 406.3	0.3 mL/min	Inken K Ramöller *et al*., [32]
LCMSMS	Phenomenex C18 (4.6×150mm)	Methanol and 0.1%HCOOH in the ratio of 81:19, v/v	(m/z) of RPV at 367.4 and CAB at 406.4	0.5 mL/min	Weld ED *et al*., [33]
UHPLC-MS/MS	HSS Xselect T3 columns from Water 2.1× 5mm, 3.5µ	Garadient (H_2O+0.1% HCOOH (A) and ACN+0.1% HCOOH(B))	(m/z) of RPV at 367.9 and CAB at 406.6	300 µL/min	Courlet P *et al*., [34]
UHPLC	UPLC Acquity BEH Phenyl columns, 1.70µm, 15 mm×2.1 mm	A: 0.5% HCOOH:ACN(94:6,v/v); B: Methanol:ACN (94:6v/v)	258 nm	0.3 mL/min	Lidija Kovač *et al*., [35]
HPLC	C18 Kromasil 150x 4.6mm, 5.0µ	KH_2PO_4: ACN (60:40)	257 nm	1 mL/min	Anugu Prasanna *et al*., [36]
HPLC	Agilent 150 (4.6 x 150mm, 5µm).	ACN: OPA taken in the ratio 54:46	257 nm	1 mL/min	Nagireddy Vasantha *et al*., [37]
HPLC	C18 (BDS) Agilent column (150X4.6mm, 5µ)	KH_2PO_4(pH4.8): ACN(7:3 v/v).	260 nm	1 mL/min	Suneetha A *et al*., [38]
HPLC	C18 Kinetex columns (250 x4.6mm, 5µm)	ACN(35) : (65) Sodium Dihydrogen Phosphate buffer (0.05M) of pH 5.5	242.5 nm	1 mL/min	Pandya, Yogi *et al*., [39]

Table 2: Reported methods for the combination of Glecaprevir and Pibrentasvir.

Method	Column	Mobile phase	Detection	Flow rate	Author
HPLC	C18 HALO, 150mm×4.6mm, 2.7µ	A: Phosphate 2.5 buffer:ACN(7:3), and B: H_2O :ACN(3:7)	252 nm	0.8 mL/min	Vemuri DK *et al*., [40]
UPLC	UPLC Acquity C18(50×2.1mm, 1.70µ)	OPA 4.2 buffer:ACN (6:4)	260 nm	0.35 mL/min	Susmita, A.G *et al*., [41]
HPLC	C18 water column 250X4.6mm, 5µm	OPA 0.5mM : ACN (75:25v/v), 4.3 pH	225 nm	1 mL/min	China Babu D *et al*., [42]
HPLC	Std Zodiac 150 x 4.6 mm, 5m.	OPA 0.1%: ACN (5.5:4.5)	260 nm	1 mL/min	Yeragodala Narendra Reddy *et al*., [43]
HPLC	RP(250X4.6mm), 5µ	phosphate pH 4.0buffer and (30:70)	251 nm	1 mL/min	Narayanaswamy Harikrishnan *et al*., [44]
HPLC	Cosmicsil, 250mm and 4.6mm	0.1M KH_2PO_4, 65 ml and methanol 35 ml	230 nm	1 mL/min	Patta Salomi *et al*., [45]
HPLC	C18 Altima column 150x 4.6mm, 5µ	Methyl alcohol, TEA and ACN in 5.5:2.5:2.0 v/v	225 nm	1 mL/min	Saradhi NDVR *et al*., [46]
LC–ESI-MS/MS	TC- Agilent C18, 4.6×75mm, 3.5µ,	ammonium acetate5 mM: ACN (2:8 v/v)	GCPR m/z 838.87-337.26, PBTR 557.51-210.40, and GCPR -13C-d7 846.91-337.26	0.5 mL/min	Konda Ravi Kumar *et al*., [47]

Table 3: Reported methods for the combination of Lamivudine and Tenofovir

Method	Column	Mobile phase	Detection	Flow rate	Author
HPLC	Thermo Hypersil BDS 120A (250×4.60mm; 5µ C18)	ACN and phosphate (PH 3.5) buffer (8:2)	260nm	1.20 mL/min	Dubbaka A *et al*., [48]
HPLC	Phenomenax Luna C18 (150mm×4.6mm, 5 µ)	ACN: methanol: H_2O(30:50:20)	258 nm	1 mL/min	Karunakaran *et al*., [49]
HPLC	Phenomenex C_{18} Luna 150mm×4.6mm, 5-µm column,	methanol: ACN: H_2O (45:35: 25)	258 nm	0.5 mL/min	Anandakumar *et al*., [50]
HPLC	(15cm x4.6mm, 5µ)C18 Inertsil column	phosphate 6.5 mM buffer and ACN (5:5)	260 nm	1 mL/min	Sonawane P.H *et al*., [51]
HPLC	Phenomenex 5µ Luna C18(250×4.6mm×5µ)	Methanol (4.6) with OPA	260 nm	0.6mL/min	Haribabu Y *et al*., [52]
HPLC	ODS- Inertsil 3V (250mm × 4.6mm, 5µ)	ACN: IPA 1%(85:15)	256 nm	1 mL/min	Rao N *et al*., [53]
HPLC	C18 Agilent (250mm × 4.6mm, 5µ)	phosphate 0.05M buffer and ACN (60:40)	260 nm	1 mL/min	Sk.Mastanamma *et al*., [54]
HPLC	C18 Symmetry (4.6x100mm,3.5µ)	phosphate 4.0 buffer and ACN (4.2:5.8)	254 nm	0.5 mL/min	VANAJA P *et al*., [55]
HPLC	C18 Ascentis (150mm × 4.6mm, 2.70µ),	buffer phosphate and ACN (5:5)	230 nm	1mL/min	Kokkirala TK *et al*., [56]
HPLC	HYPERSIL C18 Symmetry (4.6 x100mm)	methanol: H_2O (2.5) and TEA 0.1% (6.8: 3.2v/v)	260nm	1.2mL/min	Srinath A *et al*., [57]

Bioanalytical method validation

Need of bioanalytical method [58-60]

Well-characterized, validated bioanalytical procedures are needed to interpret accurate, dependable data. Bioanalytical approaches are constantly improving. It's also vital to note that bio-analytical techniques vary per analysis. Validation needs vary each research. Bioanalytical procedures must be tested at each site and in different locations for interlaboratory reliability when testing samples for a research at several sites.

Precision

The word "precision" refers to the degree to which a set of observations are gathered under particular circumstances from numerous samples of the same homogenous sample. This ensures that the results are as accurate as possible. Interday, intraday, and various analysts are the extra subcategories that are included in precision. Tests of precision or repeatability have been carried out in order to determine the degree of accuracy in time. These controls may have been implemented by the use of a variety of observers, apparatus, reagents, and labs.

Accuracy

An analytical technique is said to have high accuracy when there is a high degree of agreement between the value that was discovered and the value that was regarded as a typical, true, or mutually agreed upon reference value.

Linearity

Linearity measures the system's capacity to deliver test findings proportionate to sample analysis. Drug manufacture requires calculating the linear range. The accuracy analysis must start and finish with the five concentration levels.

Recovery study

The analytes recovered and the internal standard should be consistent, trustworthy, and repeatable, even when the recovery study cannot be faultless. Recovery tests should use unextracted parameters that demonstrate 100% reconstruction for extracted samples at three concentrations [low, medium, high].

Matrix effect

The matrix effect is the influence of a biological sample's co-eluting residual matrix on target ionization. The matrix effect may be generated by amines, urea, and carbohydrates. The variation of six-lot matrix coefficients is less than 15% of the normalized matrix factor [mf].

Dilution Effects

If the technique assesses diluted samples, the integrity of the dilution should be verified throughout validation by dilution QCs above ULOQ with similar matrices to bring them with in quantification ranges and demonstrating their precision and accuracy. Validation should match research dilutions.

Stability

Autosampler stability: Only if autosampler storage circumstances vary from extract (processed sample) stability should the sponsor show extract stability in the autosampler. Bench-top stability: The sponsor should assess sample stability under the laboratory handling settings predicted for the research samples (e.g., room temperature or ice bucket storage). • Extract (or processed sample) stability: The sponsor should test processed samples, including autosampler residence duration, against newly produced calibrators. • Freeze-thaw stability: The sponsor should test the sample after three freeze-thaw cycles. Thaw and evaluate QC samples like research samples. Freeze QC samples for 12 hours between cycles. Freeze-thaw stability Fresh calibration curves and QCs should be compared. • Long-term stability: The sponsor should evaluate the sample's long-term stability during a period equivalent to or greater than the time between initial sample collections and final samples assessment. Study temperatures should match sample storage temperatures. Compare long-term stability QCs to newly created linearity plotss and QCs. Stabilities at -20 degrees covers cold temperature. • Stock solution stability: Stocks solution should not be produced from expiring references material unless the analyte's purity is restored.

CONCLUSION

We have come to the conclusion that bioanalytical techniques are often employed to measure medicines and metabolite in physiological matrix, and these approaches may be applicable to investigations involving human clinical as well as nonhuman pharmacology and toxicology. In order to properly evaluate and understand the results of bioequivalence, pharmacokinetic [PK], and toxic kinetic investigations, it is necessary to make use of a bioanalytical technique that can measure medications and the metabolites of those drugs in biological fluids. Advantages of LCMS-MS include low detection limits, capacity to yield structural information, the need for little sample preparation, and the capability to cover a large variety of analytes with variable polarity. LC/MS/MS devices, despite their excellent sensitivity and selectivity, have several limitations owing to matrix-induced fluctuations in ionization efficiency as well as ion suppression/enhancement effects brought on by biological matrix.

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