A RESEARCH ON METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SELECTED ANTIVIRAL DRUGS IN COMBINED DOSAGE FORM BY VARIOUS ANALYTICAL TECHNIQUES

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ABSTRACT

As a direct consequence of the launch of novel medicines, more recent analytical methods need to be created for these medicines. In order to make an existing method suitable for the new applications, it was necessary to make certain adjustments to the method. The discovery of new therapeutic targets is an essential step in the manufacturing of brand-new pharmaceutical drugs. The consumption of high-quality items is a certain way to get better health results.

The purpose of antiviral medication is to shorten the length of illness as well as infectivity while simultaneously lowering the severity of symptoms. The present work provides analytical approaches that are rapid, straightforward, sensitive, and focussed for the purpose of detecting antiviral drugs that are administered in combination dosage form.

Keywords: *RP-HPLC, validation*

VIRUSES

A virus is the most pervasive and all-encompassing kind of parasite. They not only steal resources from the host cell, but they also urge the metabolic process of the host cell to make more virus particles. This is done in addition to the fact that they steal resources from the host cell. This is essential to their continued existence. Antiviral chemotherapy was not an option for treatment since in order to be effective, it would have to interfere with the normal cellular metabolism of the host.

On the other hand, research conducted over the last half century has shown that infected cells do indeed contain virus-directed enzymes. In addition, many viruses contain a limited number of their own enzymes, and it is possible that these enzymes have a greater affinity for certain inhibitors or anti metabolites than the enzymes that are present in healthy cells. Drugs have also been developed that are able to target specific processes, such as the assembly of viruses, cell penetration, uncoating, reverse transcription, or maturation of viruses.

Classification of Viral infection

There are various ways that viruses may propagate. Certain viruses can only be transmitted by close personal contact, saliva, or even the air. Two of the most common ways that some viruses might spread are via sexual contact and the exchange of used needles that may still be infectious. Ticks and mosquitoes are two examples of insects that have the potential to function as "vectors" and transfer diseases from one host to another. The consumption of tainted food or drink is one potential route via which viruses might enter the body.

- **Viral respiratory infections**

1. Rhinovirus:
2. Seasonal influenza:
3. Respiratory Syncytial virus:

- **Viral Skin Infections**

1. Molluscum contagiosum
2. Herpes simplex virus-1
3. Varicella-zoster virus

Causes of Viral infection

Once a virus has infected a host and begun to replicate inside that host, the illness becomes contagious and has the potential to be passed on to other people. Each of the several distinct types of viruses has the ability to infect humans and bring about a distinct illness or condition. It has been shown that there are over 200 different viruses that may cause upper respiratory infections, one of which being the common cold. Epstein-Barr virus, which is responsible for infectious mononucleosis, AIDS, and human immunodeficiency virus; human papillomaviruses, which are responsible for HPV infection, cervical dysplasia, genital warts, and cervical cancer; and hepatitis C virus, which is responsible for hepatitis C.

Classifications of Antiviral Agents

1	Herpes virus defense	Idoxuridine, Trifluridine, Famciclovir, Ganciclovir, Valganciclovir, Cidofovir, Foscarnet, and Fomivirsen are some examples of antiviral medications.
2	influenza vaccine	Rimantadine, Oseltamivir, Zanamivir, Amantadine
3	Hepatitis-specific and non-selective antiviral medications	
a	Mostly to treat hepatitis B	Lamivudine, Adefovir dipivoxil, Tenofovir
b	Mostly to treat hepatitis C	Ledipasvir, Sofosbuvir, and ribavirin
4	Anti-Retrovirus	
a	inhibitors of nucleoside reverse transcriptase	Didanosine, Tenofovir, Zidovudine, Stavudine, Abacavir, and Emtricitabine
b	Inhibitors of nonnucleoside reverse transcriptase	Efavirenz, Delavirdine, and Nevirapine
c	Protease blockers	Ritonavir, Atazanavir, Indinavir, Nelfinavir, Saquinavir, Amprenavir, and Lopinavir are other examples.
d	Entry (Fusion) inhibitor	Enfuvirtide
e	CCR5 receptor inhibitor	Maraviroc
f	Integrase inhibitor	Raltegravir

SOME RELATED LITERATURE REVIEW

Sunder BS et al. (2018) The measurement of ledipasvir and sofosbuvir in plasma was developed and confirmed using a unique, sensitive, and accurate high-performance liquid chromatography with ultraviolet/visible light detection (HPLC-UV/VIS) approach. The analytes were extracted using a liquid-liquid extraction technique on an Oyster BDS RP-C18 column with a mobile phase made up of acetonitrile and buffer solution, methanol, and acetonitrile in the ratio of 200:600:200 (V/V).

UV detection at a wavelength of 238 nm and a flow rate of 1.0 mL/min were used. Ledipasvir and Sofosbuvir had retention times of 4.61 and 9.09 minutes, respectively. Ledipasvir and Sofosbuvir were both shown to have linearity between 250 and 2000 ng/mL, respectively. Precision has a coefficient of variation of less than 2% both within and between days. According to USFDA requirements, the technique was verified, and the findings fulfilled the standards for acceptability for selectivity, sensitivity, linearity, precision, accuracy, recovery stability of the solution, stability of the solution in plasma, and dilution integrity.

Ganapaty et al (2018) developed stability indicating reversed-phase high-performance liquid chromatography method; validated method for simultaneous detection of sofosbuvir and ledipasvir in tablet form. An isocratic mode and a Discovery C18 column with measurements of 250 by 4.6 millimetres and a particle size of 5 were employed for the chromatographic separation. The mobile phase, consisting of 45:55 (%V/V) acetonitrile and 0.1% orthophosphoric acid at a flow rate of 1.0 mL/min and room temperature, was utilized.

The wavelength that was applied during the detecting procedure was 270 nm. Sofosbuvir's retention time was found to be 2.08 minutes, but ledipasvir's retention time was 3.06 minutes. At the concentration ranges of 100 to 600 g/mL and 22.5 to 135 g/mL, respectively, sofosbuvir and ledipasvir both displayed linear concentration-response relationships.

The method that was developed was tested, and the results demonstrated that it was precise, focused, and robust. Both medicines were subjected to a battery of demanding testing, which included exposure to conditions that were acidic, basic, oxidative, photolytic, and thermal. The results of the degradation were thought to be sufficient. The simultaneous determination of sofosbuvir and ledipasvir in tablet form may be accomplished using this method.

Identification of Drugs

All medications' physicochemical characteristics were evaluated based on their description, condition, and odour, as well as their solubility in various solvents such water, acetone, chloroform, and methanol.

By employing a melting point device to determine the melting point range and spectral analysis to compare each drug's specific IR spectrum to a reference spectrum, drugs were identified.

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Optimized Chromatographic Condition

The HPLC experimental conditions were optimized on the Cosmosil C18, (250mm x 4.6mm, internal diameter, 5µm particle size) analytical column.

Optimized Chromatographic Conditions

Parameters	Conditions
Column	C_{18} COSMOSIL, (250mm x 4.6mm, 5µm)
Mobile Phase	Acetonitrile: 0.1% OPA, (55:45) %V/V

Flow rate	0.7 mL/min
Column oven temperature	30±0.3°C
Autosampler temperature	15±3°C
Volume of injection	20 µL
Detector	PDA detector
Detection Wavelength	283 nm
Run time	10.0 minutes

Preparation of Solutions

Orthophosphoric acid in water (0.1% V/V)

In a measuring cylinder with a 500 mL capacity, pipette 0.5 mL of orthophosphoric acid into 250 mL of HPLC grade water. Fill the remaining space to 500 mL with HPLC grade water. The materials were transferred and well mixed in a reagent bottle. Kept at room temperature. Within 3 days after its preparation, this solution was employed.

Mobile phase

Acetonitrile : 0.1% OPA (55:45 % V/V) 450 mL of 0.1% OPA and 550 mL of acetonitrile were combined well in a reagent bottle before being transferred to the measurement cylinder. Kept at room temperature. Within 3 days after its preparation, this solution was employed. The diluent also included the same.

Solution for Auto Sampler Rinsing

500 mL of methanol and 500 mL of water were poured into the measuring cylinder, transferred, and well mixed in the reagent bottle. Kept at room temperature. Within 3 days after its preparation, this solution was employed.

Sofosbuvir Stock Solution, 4000 µg/ mL

A 10 mL volumetric flask containing 40 mg of standard sofosbuvir that had been accurately weighed was then filled with the necessary amount of methanol to bring the sofosbuvir concentration up to its ultimate value of 4000 g/mL. Within seven days of the preparation date, the solution was utilized inside the solution and kept in the refrigerator at a temperature of 5–3°C.

Ledipasvir Stock Solution, 900 µg/ mL

Ledipasvir standard dosage of 9 mg was accurately weighed and then transferred to a 10 mL volumetric flask. The correct amount of Methanol was then added to bring the final concentration of Ledipasvir to 900 g/mL. Within seven days of the preparation date, the solution was utilized inside the solution and kept in the refrigerator at a temperature of 5–3°C.

Method Development and estimation of Sofosbuvir and Ledipasvir

Optimized Chromatographic Condition

The HPLC experimental conditions were optimized on the Cosmosil C18, (250mm x 4.6mm, internal diameter, 5µm particle size) analytical column

Forced Degradation studies and the simultaneous determination of Lamivudine and Zidovudine

The developed RP-HPLC method was used for the estimation of Lamivudine and Zidovudine. The developed method was successfully validated as per ICH Q_2 (R1), and from the results, it was concluded that the present method might be used for the routine estimation of the raw materials and in the pharmaceutical formulations.

The stability indicating assay method was used for the analysis of Lamivudine and Zidovudine. The developed method was

successfully validated as per ICH Q_2 (R1) and from the results, it was concluded that the present method might be used for the routine analysis of the raw materials and in the pharmaceutical formulations.

The Forced Degradation study revealed that there was no interference from the degradation products at the retention time of Lamivudine and Zidovudine in acid, alkali, oxidative, photolytic and thermal degradation study.

LC-MS study was carried out to identify the analyte of interest, and from the study, it revealed that the MS spectrum of Lamivudine and Zidovudine were significantly different from the acid, alkali, oxidative, photolytic and thermal degraded products.

From the successful completion of the validation study and the results found, it was concluded that the proposed method was linear, sensitive, precise, robust and accurate for the simultaneous estimation of Lamivudine and Zidovudine in raw materials and Pharmaceutical formulation.

Usefulness: The result of the analysis of the combined mixture by the proposed method was found to be highly reproducible and reliable. The degradants present in the mixture of the assayed samples did not interfere with the determination of Lamivudine and Zidovudine. So, the developed HPLC method is linear, sensitive, precise, robust, accurate and stable and can be used for routine analysis of the combination of Lamivudine and Zidovudine in pharmaceutical dosage forms. The method was validated as per ICH guidelines.

Further Clues: The stability-indicating method and impurity profiling was done for Lamivudine and Zidovudine in combination. Still, furthermore, the scope is development and validation of bioanalytical method and pharmacokinetic parameter determination of Lamivudine and Zidovudine combination.

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