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Review Article

Analytical Method Development and Validation of Antimicrobial Agent by RP HPLC

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Abstract:

To determine thirteen specific pharmaceutical compounds (metformin, amoxicillin, chloroquine, theophylline, trimethoprim, caffeine, norfloxacin, ciprofloxacin, acetylsalicylic acid, doxycycline hyclate, metronidazole, albendazole, and cloxacillin) in bulk and tablet dosage form, this study set out to develop and validate an HPLC method. The chosen target compounds were completely separated by chromatographic separation using a Kromasil C18 column, gradient elution with aqueous formic acid (0.1%), methanol, and acetonitrile, a UV absorption wavelength of 250 nm, and a mobile phase flow rate of 1 mL/min over a 22-minute run time. The author has provided a quick overview of the history of chromatography, the many kinds of chromatographic methods, uses of chromatography, and the requirement of medicines in human existence. Additionally, the author has briefly explained the significance of analytical chemistry in the pharmaceutical sector. This chapter specifies the goal of the current study and primarily explains the RP-HPLC procedure, the processes in method development, and the validation parameters of the suggested technique

Introduction

From the moment of birth until death, human existence mostly relies on medications, including vaccinations, prescription doses, pharmaceuticals, vitamin tablets, mineral supplements, energy drinks, boosters, and so forth. The Latin term "ars medicina," which meaning "the art of healing," is where the word "medicine" originates. The medical and pharmaceutical sciences have advanced, allowing people to live longer, healthier lives. The most essential human requirement, medicines extend human life expectancy and are essential in treating illnesses and other health problems. Individuals with conditions like diabetes, hypertension, and others are entirely dependent on medications for the duration of their lives. Therefore, the mainconcerns of medicine are human health and wellbeing. Since there was little pollution, good eating practices, a lot of physical labor, less mental stress from work, and other factors during our ancestors' time, their health was not much impacted by a variety of ailments. However, today's illnesses and health issues are being caused by a number of factors, including the fast growing population, changing dietary habits, a lack of physical labor, intense job pressure, mental stress, and different types of pollution. The use of medications is

rising dramatically along with the number of ailments.

Appropriate medications or pharmacological doses in the suitable combinations are necessary for the efficient treatment of a variety of illnesses and health problems. Since ancient times, there have been medicines, and the majority of them are made using natural ingredients, herbs, and other home remedy techniques. The effectiveness of the medication and current technology are the primary considerations in the preparation of the medications. Pharmaceutical companies assess the quality of their final product and the compliance of their raw materials with their specifications through qualitative and quantitative analysis. The medicines should meet specific requirements, such as purity, effective reaction time, and lack of side effects. While quantitative analysis aids in determining the quantity of a specific element, species, or compound in the sample, qualitative analysis focuses on identifying the elements, functional groups, or compounds within the sample. This allows us to maintain the sample's allowable limits for impurities.

1.1 High Performance (or High Pressure) Liquid Chromatography - HPLC

In ion/molecular exchange chromatography, the efficiency rises when the gel matrix's size uniformity and particle size are improved. An unprecedented degree of efficiency is reached when the matrix grain size is between 3 and 10 µm. High performance liquid chromatography, or HPLC for short, is liquid chromatography carried out with the use of such resins. Nevertheless, at these particle sizes, the only way to get an adequate flow of the mobile phase (eluent) is to apply a high pressure of around 10 MPa using specialized precision pumps. Thus, high pressure liquid chromatography is sometimes referred to as HPLC. Because HPLC columns are subjected to high pressure, they must be incompressible. Under the right circumstances, silica may be utilized to produce homogenous column media with a well-regulated particle size and adequate strength.

High pressure is used by HPLC to push an analyte or sample combination through a stationary phase (chromatographic packing material)-filled column. A stream of nitrogen or helium gas is flowing while the sample is being handled. The substances in the sample that may dissolve in a liquid at trace amounts as low as parts per trillion can be identified, separated, and quantified using HPLC. Because of its versatility, HPLC is used in a wide range of commercial and scientific settings, including forensics, chemicals, pharmaceuticals, and the environment. The interaction between the stationary phase, the compounds under study, and the solvent used determines theretention durations. Analytes with varying polarities interact with the stationary phase and mobile phase at different rates during the sample's passage through the column; those with the least amount of interaction with the stationary phase or the greatest amount of interaction with the mobile phase will exit the column earlier.

1.2 Types of HPLC

- **Normal Phase HPLC**

Analytes are separated by polarity using this procedure. Polar stationary phase and non-polar mobile phase are used in NP-HPLC. As a result, silica is typically the stationary phase whereas hexane, methylene chloride, chloroform, diethyl ether, and their combinations are the mobile phases. As a result, polar samples are kept on the column packing's polar surface for longer.

- **Size - Exclusion HPLC**

The material with carefully regulated pore diameters is poured into the column, and the particles are sorted based on molecular size. Smaller molecules enter the packing particles' pores and elute longer, whereas

larger molecules are quickly washed through the column.

- **Ion - Exchange HPLC**

Ionic or ionizable samples are the only ones that may be utilized with this method. An ionically charged surface that is oppositely charged to the sample ions makes up the stationary phase. The sample will take longer to elute because of its increased attraction to the ionic surface due to its higher charge. Elution time is controlled by both pH and ionic strength in the mobile phase, which is an aqueous buffer.

- **Reverse Phase HPLC**

This technique involves a polar liquid, such as a combination of water and methanol or acetonitrile45, as the mobile phase and a non-polar (hydrophobic) stationary phase. Because it operates on the basis of hydrophobic interactions, longer-lasting materials are those that are more non-polar. Because HPLC can only be used with hydrophilic silica stationary phase, it was particularly useful for the separation of hydrophobic organic solvent-soluble compounds. Hydrophobic silica gels were later made feasible by the chemical alteration of the silica surface. The term Reverse Phase Chromatography (RPC) refers to the situation when the stationary and mobile phases' hydrophilic-hydrophobic connection was inverted.

1. Literature Review

Sravanthi, Gandu & Gandla, Kumaraswamy & Repudi, Lalitha (2023) For the purpose of estimating Molnupiravir in bulk and in its pharmaceutical dose form, a novel reversed-phase high-performance liquid chromatography approach that is straightforward, selective, quick, and accurate has been developed and verified. Using a Symmetry ODS C18 (4.6×150mm, 5µm) column, the separation was achieved. Methanol was present in the employed mobile phase. Phosphate Buffer pH-4.2 was adjusted in an isocratic manner at a wavelength of 236 nm using a 35:65% v/v solution of orthophosphoric acid. The injection volume and the mobile-phase flow rate were 10 µL and 1 ml/min, respectively. Molnupiravir was shown to have a retention time of 2.8 ±0.2 minutes. Molnupiravir had a strong linear correlation (r = 0.999) throughout a concentration range of 20 to 100µg/ml. Molnupiravir's limits of detection (LOD) and quantitation (LOQ) were determined to be 2.6µg/ml and 6.35µg/ml, respectively. The observed recovery percentage fell between 98 and 102%. For the accuracy investigation, the relative standard deviation was determined to be less than 2%. The suggested approach is ideal for the estimation of Molnupiravir in bulk and marketed pharmaceutical dosage form since it is fast, straightforward, exact, specific, and accurate. It was determined that the already established RP-HPLC technology is easy to use, quick to build, and accurate, making it suitable for regular quality control analysis in the pharmaceutical sector.

Bashimam, Mais & El-zein, Hind (2023) This study presents a novel approach to the detection and quantification of meropenem trihydrate using Reverse-Phase High Performance Liquid Chromatography (RP-HPLC), which is environmentally friendly. With a C8 column and an isocratic mobile phase (60 percent methanol and 40 percent ultra-pure water), this approach provides a quick RP-HPLC methodology that does not use buffers or acetonitrile. Traditional techniques also employ gradient mobile phases and longer retention durations. It has an impressive r^2 value of 0.9995, while having a short retention duration of 2.1 minutes. Together with low LOD and LOQ values of 1.72 and 5.20 µg/ml, it offers accuracy (101.1-102.3%) and precision (RSD < 2%). Sample preparation is made easier using aqueous dilution, which also reduces interference and deterioration. Comparing the approach to an HPTLC method reveals that it has a high sensitivity and an extended linear range (6.25-200 µg/ml for HPLC and 7.81-62.5 µg/ml for

TLC), which makes it important for meropenem trihydrate quality control in both bulk and dose forms.

Kundu, Pradeep & Pawar, Neelam (2023) The current project is to create an analytical technique and verify it in order to ascertain the antibacterial dug-in gel formulation's assay. Analytical method validation is the process of confirming through laboratory studies that a procedure, method, system, or analyst produces accurate and repeatable results and that the technique's performance characteristics meet the requirements needed for analytical applications. Analytical method validation is the process of verifying through laboratory studies that a procedure, method, system, or analyst produces accurate and repeatable results and that the technique's performance characteristics meet the requirements needed for analytical applications. Analytical method validation is the process of verifying through laboratory studies that a procedure, method, system, or analyst produces accurate and repeatable results and that the technique's performance characteristics meet the requirements needed for analytical applications.

Dharmamoorthy, G & Balaji, Anna & Vishnu, G (2022) RP-HPLC technique for the simultaneous measurement of ampicillin and cloxacillin in pharmaceutically marketed goods such as Capsule Brand No. 1 and Brand No. 2 is being developed in this study.Acetonitrile, phosphate buffer pH 5, and ampicillin are simultaneously estimated using the RP-HPLC method, with a 35:65 ratio used as the mobile phase.Column: Hypersil C18, 250 x 4.6 mm, 5µ. The results showed that the retention times for ampicillin and cloxacillin were, respectively, 3.130 and 7.907 minutes. The two peaks had high peak symmetry and form, and they were well resolved. The validation summary of the RP-HPLC technique meets all validation requirements.

Mangrio, Ghulam & Maneengam, Apichit (2022) A very accurate, smooth, highly sensitive, and highly repeatable RP-HPLC method was created and shown to be able to estimate Sofosbuvir (SOF) in pharmaceutical dose formulations. Phenomenex Luna C-18 (150 mm × 4.6 mm × 5 µm) (USA), an Agilent High-Pressure Liquid Chromatograph 1260 with GI311C Quat. Pump, and a Photodiode Array Detector (PDA) G1315D were used in this procedure. For the investigation, the cell section was used, along with acetonitrile and methanol at an 80:20 v/v ratio and solution (B) of 0.1% phosphoric acid (40:60). Nevertheless, a drift flow of 1 mL/min was used to inject 10 µL of the sample. PDA set at 260 nm was employed as the eluent, and the separation was place at a column temperature of 30 °C. Five minutes was the SOF retention period. A correlation value of 0.99 indicated that the calibration curve was updated linearly between 0.05 and 0.15 mg/mL, and there was true linear relationship between top vicinity and awareness in the calibration curve. 0.001 mg/mL was the limit for detection and 0.003 mg/mL for quantification. Between 98% and 99% of SOF was recovered from pharmaceutical components. The SOF assay value was 99%. After analyzing several analytical validation characteristics, including selectivity, accuracy, precision, linearity, and percentage relative standard deviation (%RSD), it was found that the percentage was less than 2%. Every other important parameter was found to be within the intended ranges. As a result, the suggested RP-HPLC method was successful in producing SOF in both prescription tablet dosage forms and bulk. Based on drug similarity and ADMET profiles, the computational research and drug repositioning of SOF demonstrated minimal toxicity and effective antiviral efficacy.

2. RP HPLC-Based Bio-Analytical Method Development And Doxycycline Validation In Dosage Forms

One of the most used analytical methods in chromatography is High Performance Liquid Chromatography (HPLC). A separation method involving mass transfer between the stationary and mobile phases is called a chromatographic process. A liquid mobile phase is used in HPLC to separate the constituent parts of a mixture. A liquid or a solid phase might serve as the stationary phase. These ingredients are mixed together and dissolved in a solvent before being pushed under intense pressure through a chromatographic column. The mixture splits into its constituent parts in the column. The degree of interaction between the solute components and the stationary phase determines the resolution, which is a crucial factor. The immobile packing material in the column is referred to as the stationary phase. By selecting distinct solvents and stationary phases, one may control how the solute interacts with both mobile and stationary phases. Because of this, HPLC gains a great degree of adaptability that is unmatched byother chromatographic methods and is able to separate a large range of chemical combinations with ease. A biological matrix for a chemical substance is collected, processed, stored, and analyzed using a series of steps known as a bioanalytical technique. The process of determining if a quantitative analytical technique is appropriate for use in biochemical applications is known as bioanalytical method validation, or BMV. Adopting a minimal sequence of validation experiments and attaining good results provides reassurances about the quality and dependability of the procedure. An essential part of biological assay validation is the characterization of analyte stability in biological samples obtained during clinical investigations together with that crucial assay reagent, including analyte stock solutions

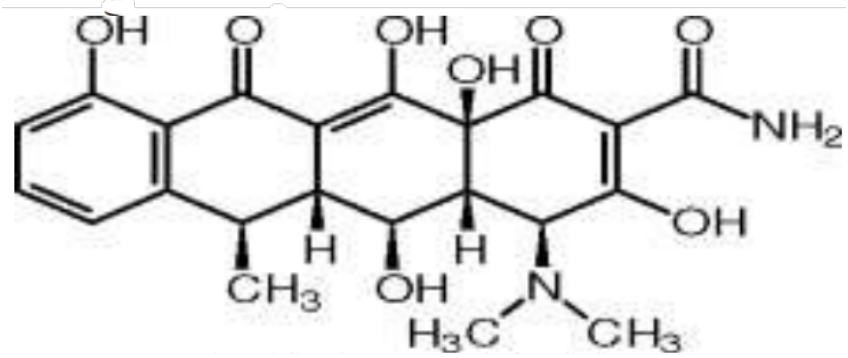

Figure 1: Chemical structure of DOXYCYCLINE

3. RP-HPLC Method For Levofloxacin And Azithromycin Simultaneous Estimation In Combined Tablet Dosage Form

Using both antibiotic and antibacterial properties, azithromycin is a macrolide antibiotic that is a member of the azalide group. Azithromycin is known by its IUPAC nomenclature as (2R,3S,4R,5R,8R,10R,11R,12S,13S, and 14R).11- {[dimethylamino)-4-(2S,3R,4S,6R)[oxy}-3-hydroxy6-methyloxan-2-yl]The trihydroxy-2-ethyl-3,4,10-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy} -13-7, 10, 12, and 14-heptamethylcyclopenta decan-15-one -1-oxa-6-aza. A synthetic chemotherapeutic antibiotic belonging to the fluoroquinolone medication class, levofloxacin hemihydrate is used to treat serious infections that pose a life-threatening risk or that do not improve with other antibiotic classes. The IUPAC name is (S).9-fluoro-2,3-dihydro (3-oxo-7H-pyrido, 7-oxo-10-(4-methyl piperazin-1-yl)[1,2,3-de]Benzoxazine-1,4-carboxylic

acid. A search of the literature found few analytical procedures for estimating azithromycin and levofloxacin3-8 alone or in combination with other medications, but there aren'tmany accessible for figuring out these medications together. Therefore, the current study's goal is to create a straightforward and focused RP-HPLC technique for the simultaneous measurement of these two medications in dose and bulk forms.

4.1 RP-HPLC Method Validation and Fast Analytical Method Development for the Accurate Estimation of Exemestane and Genistein with Particular Use in Lipid-Based Nanoformulations

Approximately 25% of women with breast cancer die away from the disease, despite significant advancements in therapy. Breast cancer has grown to be a serious worldwide health issue in recent years. Since one-third of all breast cancers are hormone-dependent and will return in premenopausal and postmenopausal women after experiencing an estrogen shortage, lowering estrogen levels is still a useful target for breast cancer therapy. The two components of the aromatase system are aromatase cytochrome P-450 and aavoprotein NADPH-cytochrome P-450 reductase. One potential treatment for hormone-dependent breast cancer is to block cytochrome P-450, which is an aromatase, in order to decrease the synthesis of estrogen by ovarian and peripheral tissue. Irreversible aromatase inhibitors are superior than nonspecific reversible aromatase inhibitors in many cases. Using the conventional catalytic mechanism, an irreversible aromatase inhibitor functions as a substrate and inactivates the enzyme's active site by covalently binding to an intermediate molecule produced by the aromatase cytochrome P-450.

The treatment of hormone-dependent breast cancer involves the use of exemestane (EXE), an irreversible aromatase inhibitor that is chemically known as 6-methylenandrosta-1, 4-diene-3, and 17-dione ($C_{20}H_{24}O_2$; 296.403 g/mol) (Figure 2). The 1, 2-double bond in the steroid molecule's ring increased the aromatase inactivator's similarity to the aromatase enzyme, indirectly increasing the therapeutic efficacy. EXE is a medication classified as BCS Class IV and has limited permeability and poor water solubility. Furthermore, patients have a side effect of bone loss, which increases their risk of osteoporosis and bone fractures. Furthermore, therapeutic resistance to aromatase inhibitors is a concerning barrier to effective breast cancer treatment

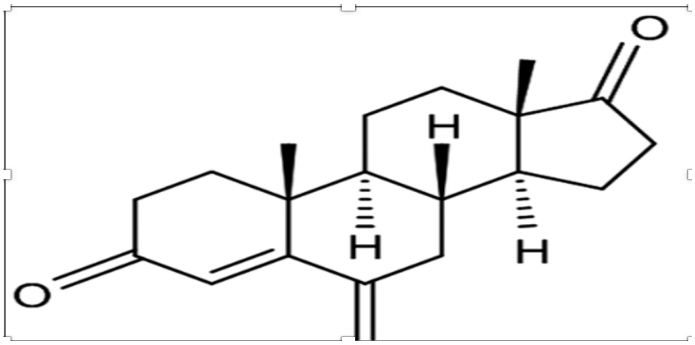

Figure 2: Chemical structure of EXE.

From this point forward, safe and effective substitute methods have been promoted to avoid the major side effects and difficulties connected with conventional chemotherapy. The combination of traditional chemotherapeutics with a herbal component will help to overcome multidrug resistance, lessen side effects, lower dosages of chemotherapeutics, and thereby increase anticancer effectiveness.

An key active component of soybeans, kudzuvine root, scoparius, and other

leguminous plants, genistein (GEN, 4′,5,7-trihydroxyisoflavone) (Figure 3) has many positive benefits on osteoporosis, cardiovascular disorders, and postmenopausal syndrome. Because of its mild estrogenic and anti-estrogenic qualities, GEN functions in the prostate, skin, and breast cancers. It does this via inhibiting topoisomerase II, inducing differentiation, activating protein tyrosine kinase, and promoting angiogenesis, among other mechanisms. Asiatic women who consume more soy products that include isoflavones have been shown to have decreased chances of osteoporosis, cardiovascular disease, breast cancer, and uterine cancer.

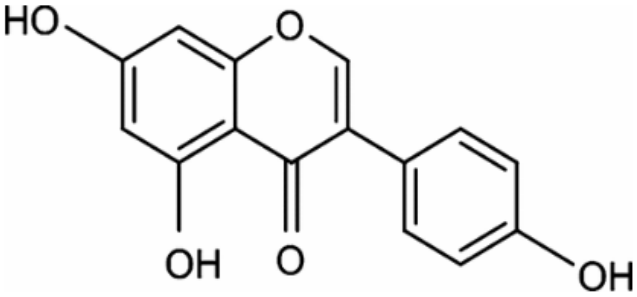

Figure 3: Chemical structure of GEN.

Considering the emerging advantages of GEN, it made perfect sense to pair it with EXE in order to achieve more therapeutic efficacy and fewer adverse effects. This new combination may be able to avoid bone hunting, one of the main negative effects of hormone treatment.

There is currently no contemporaneous technique of analysis for the combination of EXE and GEN in the literature. However, for the quantitative analysis of these two medications separately, many techniques have been used. Therefore, to progress the combination formulation, an analytical approach is needed for quality control and measurement of component medications. In order to develop and validate an RP-HPLC technique for the simultaneous measurement of EXE and GEN, the current research was developed. This technique was also used to manufacture liposomes co-loaded with GEN and EXE using the ethanol injection and rotary evaporation methods. This technique was also used to create co-loaded EXE and GEN liposomes using the thin-film hydration and ethanol injection methods. Additionally, several degradation experiments, including acid, alkaline, photolytic, and oxidative ones, were conducted to verify the method's appropriateness.

4. Use A Single HPLC Method To Determine Thirteen Pharmaceuts In Tablet and Bulk Dosage Form.

Pharmaceuticals are substances, either natural or manmade, that are widely utilized in veterinary and human medicine to prevent disease. They are also used as growth promoters, which are substances with active components intended to provide notable advantages and pharmacological effects. Following ingestion, a portion of the drugs are mostly eliminated via the urine and feces as metabolites and unaltered parent components. Pharmacological characteristics, such as therapeutic effects, may be the basis for classifying medications into different groups. The participants in this research are often administered medications that have been found in the surroundings. Antibiotics are essential pharmaceuticals that are either natural, semi-synthetic, or synthetic and are utilized as antibacterial, antifungal, or anti-parasitic medications. Amoxicillin (AMOX), a medication used to treat bacterial infections, and ciprofloxacin (CIP), a synthetic derivative of fluoroquinolone with broad-spectrum

action against several pathogenic gram-positive bacteria, are examples of commonly used antibiotics. While doxycyclinehyclate (DOXH) is a broad-spectrum tetracycline antibiotic with a wide variety of antibacterial activity, cloxacillin (CLA) is a member of the semi-synthetic β-lactam antibiotics used against staphylococci that manufacture beta-lactamas. Norfloxacine (NOR), a third-generation fluoroquinolone antibiotic, is used to treat gonorrhea and urinary tract infections as well as illnesses brought on by Campylobacter, Escherichia coli, Shigella, and Vibrio cholera. While metronidazole (MTZ) is indicated for the treatment and prevention of anaerobic bacterial infections and some bacterial illnesses, trimethoprim (TMP) is used for protozoal infections. Drugs known as anthelminthics are used to either kill or stun parasitic worms in order to expel them from the gastrointestinal system. One of the various broadspectrum benzimidazole parasiticides used to treat parenchymal neurocysticercosis is albendazole (ABZ).

Antimalarial medications, such chloroquine (CHQ), are used to treat malaria-induced fever. Adult patients with systemic and discoid lupus erythematosus, as well as rheumatoid arthritis, are taken chloroquine. Fever, discomfort, and inflammation are treated with nonsteroidal anti-inflammatory medications (NSAIDs). One of the most commonly used medications worldwide, acetyl salicylic acid (ASA), generally referred to as aspirin, is also known by its chemical name, 2-acetoxybenzoic acid. It has analgesic, antipyretic, and anti-inflammatory properties. A class of medications known as central nervous system stimulants causes an increase in both mental and physical activity. Caffeine, or 1,3,7-trimethylxanthine, or CAF, is a stimulant, psychotrope, and moderate diuretic. On the other hand, theophylline, or THP, has anti-inflammatory properties by suppressing CD4 lymphocyte activity in vitro and by releasing mediators from mast cells. Metformin (MET), an anti-diabetic drug, is used to treat diabetes mellitus II by suppressing the generation of hepatic glucose and so reducing hyperglycemia. Numerous writers have discussed the use of various analytical methods to determine medicines in pure, tablet dose form, and genuine sample. Liquid chromatography (LC) and gas chromatography (GC) are two analytical methods often used for the separation and identification of substances in mixtures. Because medicines are typically non-volatile, liquid chromatography is mostly used for this purpose. For amoxicillin, alebendazole, chloroquine, acetylsalicylic acid, caffeine, metronidazole, theophylline, and ciprofloxacin, liquid chromatographic techniques have been documented. There have been reports of spectrophotometric techniques for metformin, amoxicillin, cloxacillin, ciprofloxacin, trimethoprim, albendazole, and chloroquine. There are literature-available electrochemical techniques for metronidazole, caffeine, and theophylline; kinetic methods for amoxicillin; and chemiluminescence-based methods for norfloxacin.

As far as we are aware, no single high-performance liquid chromatography approach has been published for the simultaneous detection of these particular pharmaceutical substances belonging to several therapeutic classes. The goal of this project is to create an analytical chromatographic technique that is easy to use, precise, accurate, and stable enough to be able to separate and identify thirteen different pharmaceutical pharmaceuticals at once in a single optimized procedure, both in bulk and in commercial tablet dosage form. The ICH recommendations for the analysis have been followed in the development and validation of the suggested approach. Creating this kind of analytical technique is more economical than adjusting parameters for every analyte while analyzing actual samples. The suggested approach may be used in labs

without highly specialized, cutting-edge equipment. The chosen compounds will still be determined by the procedure with enough accuracy.

5. Conclusion

The suggested RP-HPLC technique, which is innovative, easy to use, linear, robust, rugged, highly accurate, repeatable, and requires less time, was created for the simultaneous measurement of eprosartan and hydrochlorothiazide in combination tablet dosage forms. It was shown that the average recovery percentage ranged from 98 to 100. The lack of prominent peaks in the drug chromatograms suggests that the excipients in the drug formulation do not interfere with the results. The findings met the ICH criteria and were deemed acceptable. For this reason, dissolution studies and raw material and formulation quality control are appropriate uses for the RP-HPLC technique.

References

1. Sravanthi, Gandu & Gandla, Kumaraswamy & Repudi, Lalitha. (2023). New analytical method development and validation for estimation of molnupiravir in bulk and tablet dosage form by RP-HPLC method. Cellular, Molecular and Biomedical Reports. 3. 130-136. 10.55705/cmbr.2023.375093.1087.
2. Bashimam, Mais & El-zein, Hind. (2023). Development and validation of a green RP-HPLC method for detection and quantitation of meropenem trihydrate in the bulk: A comparison with HPTLC method. Current Chemistry Letters. 10.5267/j.ccl.2023.11.003.
3. Kundu, Pradeep & Pawar, Neelam & Minocha, Neha & Poonia, Asha. (2023). Analytical Method Development and Validation for Determination of Assay of Antibacterial Drugs Besifloxacin Hydrochloride and Phenoxyethanol in Gel Formulation. Anti-Infective Agents. 21. 10.2174/2211352521666230331111623.
4. Dharmamoorthy, G & Balaji, Anna & Vishnu, G & Dheeraj, A & Narendrareddy, P & Vignesh,. (2022). Development and Validation of RP-HPLC method for the Simultaneous estimation of Ampicillin and Cloxacillin in capsule dosage form. 10.37896/YMER21.11/06.
5. Mangrio, Ghulam & Maneengam, Apichit & Khalid, Zunera & Jaffar, Tassadaq Hussain & Chanihoon, Ghulam & Nassani, Rayan & Unar, Ahsanullah. (2022). RP-HPLC Method Development, Validation, and Drug Repurposing of Sofosbuvir Pharmaceutical Dosage Form: A Multidimensional Study. Environmental Research. 212. 113282. 10.1016/ j.envres.2022.113282.
6. Singhvi, I.;Goyal,A. Visible spectro photometric estimation of aceclofenac and indapamide from tablets using folinciocalteu reagent. Ind J PharmaSci,2007,69 (1),164-165.
7. Layek, B,; Kumar, TS.;Trivedi, RK.;Mullangi, R.;Srinivas, NR. Development and validation of a sensitive LC-MS/MS method with electro spray ionization for quantitation of rhein in human plasma:application to pharmacokinetic study. J Biomed Anal,2008,2(6): 616-624.
8. Alberti, MB.;Bartolucci, G.;Bruni, B.;Coran, SA.;Vaira, MD. Physicochemical and structural characterization of diacerhein. J Pharma Biomed Anal,2009, 49,1065-1069.
9. Chaudhari, A.;Golak, M.;Deo, A.; Krishna, V.;Agrawal, H.;Peshawe, U.;Gawande, A.;Srinivas, S.; Mane, S.;Jadhav, D. Isolation and structural elucidation of two impurities from a diacerein bulk drug.J Pharma Biomed Anal,2009,49,525-528.
10. Kost RG, Hurley A, Zhang L, et al. Open-label phase II trial of amprenavir, abacavir, and fixed dose of zidovudine/lamivudine in newly and chronically HIV-1-infected patients, J Acquir Immune Defic Syndr, 2001; 26: 332-339.