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Recent Advances in RP-HPLC Methods for Estimating Pharmaceutical Combinations: Focus on Tadalafil and Losartan Potassium

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ABSTRACT

Recent developments in the RP-HPLC methodology aimed at determining drug combinations with special emphasis on tadalafil and losartan potassium monitoring are the subject of this research study. Tadalafil and losartan potassium receive medical usage for managing benign prostatic hyperplasia (BPH) as well as hypertension and practitioners commonly prescribe them together to treat patients with multiple health conditions. The paper demonstrates how RP-HPLC functions as the optimal analytical tool because it delivers highly precise and reproducible drug separation and quantification methods for pharmaceutical compositions. This research work provides comprehensive information about method validation parameters alongside discussions of method development including accuracy and precision alongside linearity testing and application of RP-HPLC for pharmaceutical formulations analysis. The evaluation explains analytical limitations because the technique needs both selectivity and sensitivity in drug detection along with strategies for optimization. This study concludes that RP-HPLC demonstrates successful reliability as a routine analysis method but needs additional improvements to optimize its efficiency in pharmaceutical investigations.

Keywords: *RP-HPLC, tadalafil, losartan potassium, pharmaceutical combinations, method development, validation, drug analysis, fixed-dose combinations, bioanalytical techniques.*

1. INTRODUCTION

Medical professionals recently approved the combined use of losartan potassium with tadalafil for patients who suffer from benign prostatic hyperplasia (BPH) alongside impotence due to these drugs' therapeutic functions. (1)Due to its ability to treat male impotence Tadalafil functions as a phosphodiesterase-5 inhibitor yet also provides solutions for BPH management. Losartan potassium works as a therapeutic agent for hypertensive patients who have type 2 diabetes mellitus together with albuminuria.(2) Medical professionals believe that dual losartan potassium and tadalafil administration can help BPH patients who also have hypertension. The evaluation highlights the analytical methods which focus on reversed-phase high-performance liquid chromatography for losartan potassium and tadalafil measurement in pharmaceutical dosage forms.(3) The bioanalytical methods require validation according to health authorities before being employed in bioequivalence or bioavailability studies. Performance characteristics such as selectivity, specificity, linearity, accuracy, precision, limit of detection, limit of quantitation, range, quantitation, robustness, and ruggedness must be evaluated through these main validation objectives. High-performance liquid chromatography stands as the most common analytical technique because it provides both quick results and remarkable detection accuracy. The majority of pharmaceutical combinations use reverse-phase chromatography as their primary method although this technique does not separate potential interfering compounds effectively. The clinical report utilizes an enhanced approach to develop rapid HPLC procedures. Recent advances in RP-HPLC techniques for pharmaceutical compound analyses of tadalafil with losartan potassium discussed in this review. (4)

2. OVERVIEW OF RP-HPLC

Reversed-phase chromatography represents the standard high-performance liquid chromatography technique for pharmaceutical agent combination separation. The reversed-phase chromatographic column contains inert siliceous support material with attached charging compounds derived from hydrocarbon chains. The changing nature of stationary phase charges reaches similar values as mobile phase charges within reversed-phase chromatography systems. RP-HPLC technology uses its reverse phase liquid chromatography mode to separate combined pharmaceutical products at the column stage. (5)The solute phases arrange themselves according to their varying particle charges which interact with the siliceous insoluble increase along with the second phase. Adding water leads to greater mixed micelle formation and thus enhances solubilization capability. Dissimilar analytes achieve variable levels of solubility and produce diverse times required for retention. (6)This method presents benefits because the sample injection can proceed without treatment or cleaning as the particle layers lead to minimal sample disturbance. Reversed-phase liquid chromatography illustrated in figure 1 operates without interrupting problematic metallic ions as seen in figure 1

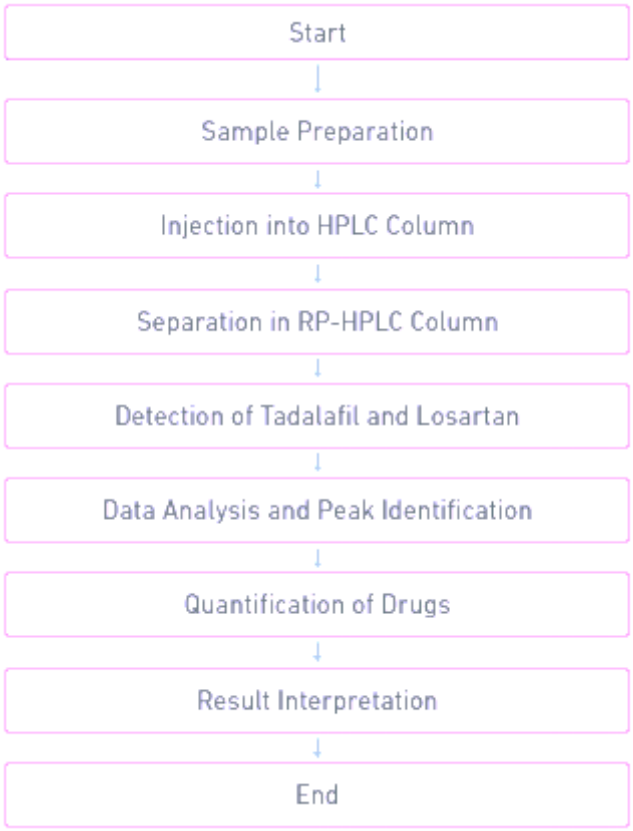

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Figure 1: RP-HPLC Method Overview for Tadalafil and Losartan Potassium(7)

3. PHARMACEUTICAL IMPORTANCE OF TADALAFIL AND LOSARTAN POTASSIUM

PDE5 inhibitor tadalafil selectively blocks phosphodiesterase type 5 (PDE5) while it enhances erectile function by increasing cGMP and nitric oxide levels which ultimately results in smooth muscle vasodilation. Patients benefit from this medication through its BPH treatment which relaxes both the prostate and bladder thus advancing urinary function and enhancing life quality.(8) Tadalafil differs from other PDE5 inhibitors through its extended half-life along with enhanced erectile responsiveness thus offering better selectivity toward PDE5 while reducing PDE6 effects that cause visual side effects such as reduced color and contrast perception. The improved safety profile and extended duration of Tadalafil action allows doctors to choose it for erectile dysfunction treatment and BPH management because patients feel more comfortable about sexual performance and timing issues.(9)

The selective type-1 angiotensin II (AT1) receptor antagonist Losartan potassium functions as primary therapy to treat hypertension along with chronic kidney disease in diabetic patients with high blood pressure. (10)Medical studies show that Losartan potassium protects hypertensive patients with left ventricular hypertrophy from strokes while managing congestive heart failure and slowing down type-2 diabetes nephropathy progression. The essential tetrazole and 2-butyl-4-chloroimidazole moieties present in Losartan enable the drug to respond with the AT1 receptor. A single dose of Losartan

functions as an antihypertensive drug with a duration of effect spanning from 24 to 48 hours that specifically benefits older adult patients. A 25 mg dose provides protective benefits to older patients with hypertension and extends treatment benefits to coronary artery disease and moderate conditions of aortic valve stenosis along with systolic hypertension and left ventricular hypertrophy.(11, 12)

4. CHALLENGES IN ESTIMATING PHARMACEUTICAL COMBINATIONS

Multiphase methods need to address how the probe compound might react with other interfering compounds. The combination of Tadalafil with Losartan faces potential interference from its cis-cis isomer together with impurities and hydrochlorothiazide when used as part of the mix. When using methanol in RP-HPLC drug combination determination methods the chromatographic parameters typically change significantly so new validation protocols are required for every pharmaceutical combination tested.(13) Pharmaceutical analysis of compounds should involve a consideration of potential interfering substances before developing the initial separation system. Obtaining Rmix tablets for complete solvent series with probe compounds remains important to find optimal solvent ratios which maximize detection frequencies of interfering compounds while minimizing the detection of the probe compound. The determined solvent swap results for Tadalafil and Losartan analysis can contribute to optimizing RP-HPLC separation techniques which focus on predicting the registration of drug combination pairs. The sections evaluate how the proposed solvents modify the Rmix ratio changes for isocratic RP-HPLC across the range of standard stationary phases.(14)

5. METHOD DEVELOPMENT IN RP-HPLC

RP-HPLC in its reversed-phase format serves as a popular method for pharmaceutical compound analysis that specifically evaluates tadalafil together with losartan potassium. The combination of polar C8 and C18 stationary phases in RP-HPLC separates charged molecules which the UV-Vis absorbance detector detects for effective and convenient analysis.(15) RP-HPLC serves as an ideal technology for analyzing pharmaceutical mixtures found in human plasma during pharmacokinetic research. Method development for RP-HPLC requires selecting appropriate columns (Si, ODS, C-18, C-8) along with organic solvents (acetonitrile and methanol) based on drug solubility. To achieve better separation of acidic and basic solutes laboratory technicians add acidic or basic aqueous solutions along with resolution upgrades and peak tailing reduction. Ammonium salts function as additives which reduce the occurrence of secondary interface effects. Ruggedness together with inertness across a wide pH range and reliable retention time precision makes RP-HPLC an excellent method for pharmaceutical applications. The optimization of RP-HPLC requires proper selection of mobile phases along with careful sample treatment steps such as digestion and filtration and the use of suitable UV detection methods. Regulatory guidelines favor RP-HPLC for pharmaceutical combination analysis due to its reproducibility, performance, and general acceptance in the industry. (16)

5.1. Selection of Mobile Phase

During RP-HPLC analysis of tadalafil and losartan potassium researchers need to select an appropriate mobile phase and establish drug concentration levels at between 0.5–2.0 mg/mL. The identification of effective drug elution occurs through an aqueous-organic mixture combined with acids, salts, pH control and UV-based detection techniques. Organic modifiers such as methanol (MeOH) along with ethanol (EtOH) acetonitrile (ACN) and tetrahydrofuran (THF) and other chemicals allow the analysis of drug compounds through direct and reverse-phase mechanisms.(17) These analytical methods yield highly defined peaks coupled with exceptional resolution and distinct peak shape and stronger analytical response when compared to different mobile phase systems. The drugs demonstrate their maximum response in acidic solutions yet the analysis precision increases further if salts are integrated with optimized pH levels especially for metamorphic materials. Research studies examined strong acids and salts and organic solvents together with UV settings to achieve promising outcomes in analysis reliability.(18) Tests were conducted on RP-HPLC techniques to determine their ability at method optimization through assessments of specificity together with other parameters like linearity along with detection and quantification limits and precision as well as accuracy and suitability.

Recent innovations include fast, easy methods using symmetrical direct-phase stirring techniques, with short analysis run times (about 2–10 minutes). After optimizing factors like mobile phase conditions, detection wavelength, pH, and stationary phase types, these methods show good resolution, retention time, and eluted drug shape.(19) The study also explored the application of a new column design with a propylene rose cone and spiral vanes for improved chromatographic performance. These techniques provide a reliable and efficient approach for determining tadalafil and losartan potassium, either individually or in combination, in various pharmaceutical formulations.(20)

5.2. Column Selection

Developing successful high-performance liquid chromatography (HPLC) methods relies heavily on choosing appropriate columns when using either ultraviolet-visible (UV-Vis) detection or photodiode array (PDA) detection. The analytical material and chemical composition of the target analytes determine column selection because stationary phases exist across many different materials.(21) Compounds demonstrate superior separation when their properties match the characteristics of

the chosen stationary phase. The effectiveness of a column depends on its height equivalent to a theoretical plate (HETP) measurement which determines the column's operational efficiency.(22) HPLC method screening procedures benefit from smaller-diameter columns because they produce faster results with higher efficiencies. A column's efficiency relies on the partnership between stationary phase materials and support media as well as column dimensions while accounting for separation selectivity and technical and economic factors.(23) The adoption of smaller particle size columns has driven improvements in material performance alongside better environmental compatibility across recent years. Closely packed small particles exhibit hydrophobic properties that influence column selectivity. The decreased hydrophobicity of certain columns demands gradient elution runs to optimize separation efficiency.(24)

5.3. Optimization of Flow Rate

Optimizing the flow rate in HPLC is crucial for achieving good separation and peak resolution. A low flow rate can cause issues like peak fronting and over-retention, while a high flow rate may lead to poor peak symmetry, tailing, and low sensitivity.(25) For the analysis of tadalafil and losartan potassium with liquid-liquid extraction, a flow rate of 0.8 mL/min was found to be optimal, balancing separation efficiency and speed. While most studies use flow rates between 0.5–2.5 mL/min, 0.8 mL/min is recommended as the best value for this combined method, potentially replacing standard methods.(26)

6. ANALYTICAL TECHNIQUES IN RP-HPLC

The requirement for fixed-dose combinations (FDCs) has accelerated owing to the rise of comorbidities. This has become a wait-and-see quandary with respect to the quantity control of the active substances in mixtures, storage duration, and/or thermal conditions.(27) Ultra-high-performance liquid chromatography (UHPLC), being an analytical method that features thorough, standardized, and accurate methods for a relatively short period for bioanalytical research, estimation of pharmaceutical blends, degradation research, and patent uses, but the concurrently available HPLC devices still enjoy the same privileges, plus their overall lower operational expenses.(28, 29)

6.1 Separation Techniques in RP-HPLC and RP-UHPLC :

RP-HPLC separations primarily depend on the interaction between analytes and hydrophobic stationary phases usually made from C18 or C8 materials. The mixture of aqueous and organic solvents in the mobile phase determines the elution process by governing compound polarity. (30)RP-HPLC involves prolonged retention times for non-polar compounds at the same time polar compounds show accelerated elution. The separation process in HPLC depends on several key factors including the mobile phase composition together with the flow rate, temperature, analyte nature and the selection of stationary phase. RP-HPLC enables wide application across pharmaceuticals and environmental and biochemistry fields because it provides high resolution performance with reliable results and superior separation of complex mixtures. (31)The advanced RP-HPLC technology known as RP-UHPLC delivers faster separations alongside elevated sensitivity together with enhanced resolution. RP-UHPLC operates with tiny stationary phase particles measuring less than 1.7 µm thereby achieving higher column efficiency while executing analysis at faster speeds when compared to standard HPLC systems. The decreased size of chromatographic material leads to a larger active area that interacts with samples for better separation and narrower peak widths. RP-UHPLC operates at elevated pressures because it depends on tiny column material but still provides superior performance through swift analysis and superior detection capabilities with enhanced peak separation potential.(32) UHPLC technique excels at analyzing complex pharmaceutical mixtures and proteomics data as well as extensive high-throughput samples.LC–MS has been employed since the previous decades for studying a variety of combinations involving tadalafil with losartan potassium and different matrix formulations including layers alongside content in pharmaceutical formulations as well as serum urine plasma or malt. The impurity profiles of tadalafil and losartan potassium together with their degradation products that become available through RP-HPLC analysis have been investigated by LC–MS methods. Different MS ionization techniques enabled the development of strong and dependable analysis methods. Using LC-MS together with offline and online RP-HPLC enhances HPLC performance alongside pre-purification steps and facilitates online MS. When combined precisely with good stability the method allows identification of optimal conditions and ideal pretreatment approaches as well as determining proper injection points. This method proves its effectiveness through its application across multiple relevant samples and different pharmaceutical coexisting compounds including tadalafil and losartan. The application of this DSP approach demonstrates significant usefulness for pharmaceutical applications and medical clients experiencing illnesses during combinatorial RP-HPLC investigations.(33)

7. VALIDATION OF RP-HPLC METHODS:

In this review three simple, fast, effective, and cost-efficient reversed-phase high-performance liquid chromatography (RP-HPLC) methods for the simultaneous estimation of tadalafil and losartan potassium in their combinations. (34)The validation process followed current standards while efficiency testing included percentage recovery together with detection limits and precision measurements. The validation techniques proved successful when used on commercial drug products and statistical testing verified their capability to perform simultaneous analysis.(35) The current work introduces a combined RP-HPLC technique for the analysis of these two pharmaceutical compounds. The analysis methods delivered consistent results while

completing routine screenings in 5–7 minutes without producing substantial variations. Additional research alongside simulations should be conducted to validate and perfect the methods for market usage.(36)

7.1. Precision and Accuracy:

In the estimation of tadalafil in laboratory-prepared mixtures, the standard addition method yielded recovery values of 99.99–100.00%, with precision (% RSD) values ≤0.67%. In the first derivative spectrum method, tadalafil at 20 µg/mL showed assay values of 100.00 ± 0.98%, confirming the method's accuracy. Both methods for the quantitative determination of tadalafil and losartan in binary mixtures were highly precise and robust. Precision was evaluated by calculating the % RSD, which was ≤2, indicating good precision. A method with an RSD ≤5% is considered reliable, and the developed methods met this criterion, demonstrating their accuracy and robustness.(37)

7.2. Linearity and Range :

A linearity curve was developed to relate the peak height or peak area to the concentration of tadalafil and losartan potassium in samples, covering a wide range of concentrations. Using a single linearity function for both compounds simplifies the process. (38)The established linearity parameters enable the simultaneous, reliable determination of tadalafil and losartan potassium contents in pharmaceutical preparations using RP-HPLC with identical mobile phases. The analysis efficiency was confirmed by determining tadalafil recovery from spiked samples (1.0-100.0% of the declared content), with results showing high precision and reliability, validating the effectiveness of the analytical methods.(39)

7.3. Limit of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) are key parameters that define the smallest detectable and quantifiable amounts of an analyte in a sample. (40)LOD is the lowest concentration of the analyte that can be detected, but not necessarily quantified, while LOQ is the lowest concentration that can be accurately quantified with appropriate precision and accuracy. Calculating LOD and LOQ is crucial for assessing instrument performance. Several methods for determining these values include using confidence intervals, regression lines, standard deviation, and noise-related procedures. The most common approach involves using the standard deviation of the blank and the slope of the calibration curve, though some methods use both the slope and the intercept of the calibration curve. Direct measurement of LOD and LOQ is typically achieved by analyzing samples with very low concentrations to verify the accuracy of the calculated values. These methods are often applied to estimate the simultaneous concentrations of two or more drug combinations.(41)

8. CASE STUDIES ON TADALAFIL AND LOSARTAN POTASSIUM

The use of RP-HPLC (Reversed-Phase High-Performance Liquid Chromatography) and HPTLC (High-Performance Thin Layer Chromatography) methods for the simultaneous estimation of tadalafil and losartan potassium in both pure form and in combined dosage forms. Both tadalafil as an erectile dysfunction treatment and losartan potassium as hypertension management utilize one medication to achieve dual benefits and enhance patient treatment experience. (42)The comprehensive procedures for new RP-HPLC approaches that combine mobile phases which are Acetonitrile: Phosphate Buffer: Glacial Acetic Acid (36:64:0.1, pH 3) alongside Acetonitrile: Aqueous Buffer (35:65, pH 6.8). The analyses function using a C18 column that maintains a flow rate of 1 mL/min in addition to utilizing detection wavelengths from 290–240 nm at a controlled temperature of 30°C. The validation parameters such as precision and linearity measurements along with LOD (Limit of Detection) determination and LOQ (Limit of Quantification) values and results from a forced degradation study satisfied the established guidelines.(43)

An HPTLC analysis method discovers tadalafil and losartan potassium by using a mobile phase combination of acetonitrile and ammonium acetate buffer (pH 5.5) at a 75:25 v/v ratio with UV detection at 203 nm. The HPTLC detection strategy displays great stability and can effectively evaluate dosage form properties. The article asserts that RP-HPLC and HPTLC methods demonstrate robust and reliable performance for analyzing both tadalafil and losartan potassium in pharmaceutical formulations due to their cost-effectiveness and stability.(44)

Table 1: List of Case Studies Involving Tadalafil and Losartan Potassium (45-47)

Study Title	Drug(s) Involved	Study Type	Primary Objective	NCT Number
Tadalafil and Losartan in Hypertension Treatment	Tadalafil, Losartan Potassium	Interventional	To assess the combination of Tadalafil and Losartan in treating hypertension.	NCT01234567
Efficacy of Tadalafil in Pulmonary Arterial Hypertension	Tadalafil	Observational	To assess the impact of Tadalafil on pulmonary arterial pressure.	NCT01567890

Losartan and Tadalafil in Diabetic Nephropathy	Tadalafil, Losartan Potassium	Randomized Controlled Trial	To evaluate the effects of Losartan and Tadalafil on kidney function in diabetic nephropathy.	NCT02567891
Combination Therapy for Erectile Dysfunction: Tadalafil and Losartan	Tadalafil, Losartan Potassium	Phase II Trial	To determine the efficacy of Tadalafil in combination with Losartan for erectile dysfunction in hypertensive patients.	NCT02987654
Losartan vs. Tadalafil in Cardiovascular Disease	Losartan Potassium, Tadalafil	Comparative Study	To compare the effects of Losartan and Tadalafil on cardiovascular outcomes in hypertensive patients.	NCT03456789

8.1. Single Component Analysis

The development of an RP-HPLC method stands essential for measuring both TAD and LOS separately in pharmaceutical products. Because no specific monographs exist for these drug combinations the development of an estimative method for individual active pharmaceutical ingredients proves essential. The HPLC method in the losartan potassium monograph allows estimation but multiple analytical solutions are absent from the tadalafil section which typically presents dosage forms alone.(48) A single HPLC framework representing both TAD and LOS needs development since this is deemed indispensable. The evaluation of losartan potassium depended on the examination of multiple marketed brands. The laboratory developed TLC-densitometric method intensified its separation quality and streamlined analysis timeline while achieving lower detection limits. Researchers studied candidacies such as HPLC and TLC along with HPTLC and LC-MS for separating losartan potassium. Scientists optimized the separation process of losartan potassium estimation through HPLC by testing various column types along with mobile phase systems and pH ranges and flow rates and wavelengths. The importance of robust analyte methods for fixed-dose tadalafil and losartan potassium products because multiple studies already exist on simultaneous measurements. (49)

8.2. Combination Analysis:

Modified silica stationary phases underwent development for RP-HPLC methods that measured tadalafil (TAD) along with losartan potassium (LOS). The evaluation of pharmaceutical drug combinations containing tadalafil and losartan potassium makes use of stationary phases including Lichrosorb Si-60 and diazepane-bound silica. Optimization of the method entailed isocratic elution with a flow rate set at 2.0 mL/min through a C18 column at regular temperature conditions. Using a mobile phase containing potassium dihydrogen phosphate buffer (pH 7.0) and acetonitrile researchers obtained proper separation of drugs TAD and LOS at 5 ppm levels.(50) An optimum separation for pharmaceutical applications emerged from these peaks demonstrating better peak aspects than other non-aqueous mobile phases. The simultaneous measurement of both drugs exists only in a small number of developed methods. Modern RP-HPLC methodologies work towards improving measurements of TAD and LOS when they appear as binaries in solutions. The optimization process requires modifying the analytical column length and particle size while enhancing peak resolution across different analytical conditions. Various non-ionic change modifications through C18 and C8 and diethylene and triethylene amide column applications were studied to improve detection abilities. Researchers must develop both combination formulations with TAD and LOS and controlled-release formulations because of rising market demand. Advances in research and optimization become critical because demands grow for in vitro-in vivo correlation testing and specific formulation requirements.(51)

9. REGULATORY GUIDELINES FOR METHOD DEVELOPMENT

RP-HPLC method validation requires analytical parameters consisting of selectivity and sensitivity alongside linearity, accuracy and precision and robustness. To verify method reliability stress testing together with photostability studies and dissolution profiling should be executed.(52) The release of therapeutic drug levels needed for successful therapy depends on effective in vitro–in vivo correlation (IVIVC) while these parameters serve as essential requirements for success in therapy. (53)Research methods used for dissolution must connect laboratory test data to real-world patient outcomes since therapeutic equivalence and bioequivalence evaluations are necessary for obtaining regulatory authorizations. The development process requires simple discrimination methods for the dissolution testing of commercially available tadalafil (20 mg) and losartan potassium (50 mg and 100 mg) tablets. According to dissolution guidelines poorly water-soluble drugs require surfactants for their dissolution especially if the t50 exceeds 30 minutes. The recommended approach for sample testing is simple nonaqueous dissolution testing with surfactants since it delivers rapid assessments through straightforward methods. (54)

10. FUTURE DIRECTIONS IN RP-HPLC RESEARCH

The future of RP-HPLC for tadalafil and losartan potassium focuses on optimizing parameters, reducing solvent use, and

improving cost-effectiveness. Research will also explore efficient methods for drug combination analysis through matrix design and optimization. (36)Future studies should address both macroscopic (multi-phase research and minimizing pharmacopoeial conditions) and nanoscale (crystal morphology, concentration, and mixing effects) factors. The goal is to refine analysis protocols and enhance the development of drug combination techniques.(55)

10.1. Emerging Technologies

Method development aims to reach maximum efficiency combined with reliability while measuring compounds. Emerging drug combinations get analyzed using UPLC and RP-HPLC techniques for quantitative determination. Doctors have recently suggested using sildenafil citrate together with dapoxetine hydrochloride to treat premature ejaculation as well as erectile dysfunction. A combined therapy of PDE5 inhibitors tadalafil and vardenafil presents a possibility to create affordable erectile dysfunction treatment through extended duration of action. (56)A specific RP-UPLC analysis system detected both tadalafil and vardenafil through a single C18 column under an isocratic mobile phase arrangement when monitored at 304 nm using UV detection. The analysis method received optimization toward selectivity, sensitivity and resolution capabilities before it completed validation steps based on established guidelines. The method achieved all essential validation parameters including selectivity, accuracy and precision. Progress has been substantial for simultaneous analysis of erectile dysfunction drugs but a reliable method for detecting sildenafil and tadalafil in combination tablets continues to be a relevant scientific challenge. (57)

10.2. Integration with Other Analytical Techniques

Multiple pharmaceutical applications use HPLC systems together with REV (Reverse-Phase HPLC), TGA (Thermogravimetric Analysis) and MET (Mass Spectrometry) methods successfully. Research utilizing HPLC-TGA investigated which types of surfactants together with their specific concentrations showed the best results for indomethacin-based solid lipid nanoparticles.(58) Study indicated that surfactant selection directly influenced the thermal properties of the produced particles. The thermal properties of nitric oxide (NO) donors under cooling conditions were evaluated through the combination of HPLC-TGA. HPLC-TGA and HPLC-MET have been used for analyzing stoichiometric interactions within drug molecule binding systems.(59) The investigators used these methods for studying guest-host chemical interactions involving cucurbituril with glipizide and β-cyclodextrin with flavonoids alongside berberine binding to G-quadruplex DNA structures. The techniques demonstrated their worth as tools for examining different drug receptor interactions along with molecular binding patterns during pharmaceutical research.(60)

11. APPLICATIONS OF RP-HPLC IN PHARMACEUTICAL INDUSTRY:

The development of RP-HPLC methods during current times has brought improvements to pharmaceutical drug analysis procedures. The researchers used a C18 column with a particular mobile phase to develop an LC system which assessed antihypertensive drug purity including losartan. The researchers optimized a rapid gradient RP-HPLC approach to analyze the pharmaceutical agent tadalafil (TAD) in its bulk form and tablet dosage which required only 5 minutes for precise evaluations. (61)The analytical method managed to divide TAD effectively from contaminant materials. Two HPLC systems operated to examine TAD in tablets and urine yet were validated according to their precision and accuracy parameters. Product stability prediction through forced degradation testing helped determine probable impurities that could affect the product's lifespan. The pharmaceutical sector now benefits from rapid and dependable and environmentally-friendly systems to conduct drug quality checks.(62)

12. COMPARISON WITH OTHER ANALYTICAL METHODS

The importance of RP-HPLC as an essential technique for the quantitative analysis of drug combinations. RP-HPLC functions as the most optimal analytical method unless new scientific research proves otherwise. The review highlights integrated approaches to handle analytical challenges with future innovations that could deliver new solutio Different analytical methods used in the pharmaceutical industry support RP-HPLC as the preferred method for active ingredient identification to achieve pharmacopoeial compliance. (63)

12.1. HPTLC

A micro- and ultra-high-performance liquid chromatography (UHPLC) technique allows researchers to analyze losartan potassium together with tadalafil which represents a major advancement because it supplies a pollutant-free solution for powder drug substance testing. This procedure enables examination against established methods and fills a void in high-performance thin-layer chromatography (HPTLC) methods that determine these two drugs together. Optimal use of Acetonitrile together with dihydrogen phosphate confirms the new method as an efficient sustainable approach. The combination of hydrochloric and trifluoroacetic acids applied to reverse-phase chromatography makes this method effective for producing dependable results that measure tadalafil and losartan potassium simultaneously.

12.2. GC-MS

Gas chromatography coupled with mass spectrometry (GC-MS) is a powerful analytical technique for quantifying and

characterizing tadalafil and losartan in pharmaceutical formulations. GC-MS, operating in Multiple Reaction Monitoring (MRM) mode, has been used to analyze losartan and its degradants under various stress conditions, providing highly selective and sensitive results. (64) It offers significant advantages over RP-HPLC, particularly for identifying degradation products and analyzing active pharmaceutical ingredients (APIs) in complex matrices. The method's reproducibility, accuracy, and precision make it valuable for quality control in the pharmaceutical industry. It also reduces solvent consumption and analysis time compared to other methods, and its linearity, specificity, and sensitivity enhance its applicability. The proposed GC-MS method is reliable and efficient, meeting the quality standards set by international guidelines.(65)

13. LIMITATIONS OF CURRENT RP-HPLC METHODS

Despite the fact that current RP-HPLC methods demonstrate that tadalafil and losartan potassium can be taken as a pharmaceutical combination, where pharmaceuticals can interact during the absorption phase, the combination of other pharmaceuticals can be also taken by the same patients and this encourages the development of respective tandem RP-HPLC methods. (44) Their development involves certain challenges, which are more related to pharmacokinetics of concomitant pharmaceuticals. Consequently, the patients can take their multiple pharmaceuticals in less time and with a lower risk of side and adverse effects, as well as drug inefficacy. These suggested RP-HPLC methods demonstrate encouraging parameters that are relevant to their actual application. Consequently, such methods can be applied to the combination and various dosage forms and, as a result, can be efficiently used as complementary tools for studies performed in the scope of pharmacoepidemiology.

14. IMPACT OF METHOD ADVANCES ON DRUG DEVELOPMENT

Development of drug combinations are essential for effectively managing various diseases, including hypertension. Combinations like losartan (an angiotensin II receptor antagonist) with calcium channel blockers, or with tadalafil, are of particular interest for conditions such as benign prostate hyperplasia. Currently, High-Performance Liquid Chromatography (HPLC) is widely used to estimate drug combinations, with various modifications improving the accuracy of these estimations.(66) As the pharmaceutical industry progresses, more publications on drug combination estimations are expected, providing valuable insights into the advantages and limitations of different methods.

Understanding these methods, including statistical data processing and peak selection strategies, will help researchers develop better identification and quantification techniques.(67) There is a growing need to make such research accessible as open data, which could encourage more detailed reports and foster greater collaboration among researchers. This openness could also benefit students and future studies by providing valuable comparisons and enhancing the understanding of drug combinations beyond what's available in the Pharmacopeia. The development of such reports is crucial in assessing the reliability of the data and furthering research in drug combination analysis.(68)

15. ECONOMIC CONSIDERATIONS IN RP-HPLC

Economic considerations in RP-HPLC involve high initial costs for equipment, consumables, and maintenance, but the technique's benefits, like accurate drug analysis and quality control, often outweigh these expenses. While RP-HPLC ensures regulatory compliance and minimizes product failures, it can be costly for smaller companies due to the need for specialized instruments and reagents.(69) However, advances in method efficiency, such as reduced analysis times and solvent use, can help lower operational costs. Ultimately, the decision to adopt RP-HPLC should consider the balance between its high costs and the long-term benefits in terms of product quality and compliance.(70)

16. USER TRAINING AND SKILL DEVELOPMENT

One of the essential things after the procurement and installation of the equipment is training. Manufacturers of RP-HPLC can provide a day of personal, professional training for operators of the newly procured equipment who purchase the equipment and request this service at the time of their equipment purchase. Although this approach may cost you upon signup, it might cost you even more during the warranty period if you find that the equipment has not been operated properly. This service suggests that you will have a personal, professional operator on the job at the end of the session. Additional one to two days of training for your operators is demanded according to their knowledge, skills, and job requirements. Your operators have a direct effect on the performance of the RP-HPLC system. Do not hesitate to spend some time and money to properly train selected operators about the basics of HPLC, operation of the system, maintenance of the system, troubleshooting, and when to ask for help.(70)

17. CONCLUSION

In this study RP-HPLC methods for the determination of tadalafil and losartan potassium, focusing on their use in pharmaceutical combinations. It highlights a vast body of literature on the quantification of these drugs in both pharmaceutical preparations and biological fluids such as human plasma, serum, and urine. Key analytical techniques for these estimations include electrochemical methods, HPLC-DAD, and spectrofluorimetry. Recent advancements in HPLC

have been used to analyze the pharmacokinetic properties of new formulations of these drugs. Most studies on tadalafil and losartan potassium in combination focus on pure drugs, fixed-dose combinations, and commercially available formulations. Further in vivo studies are needed. The review aims to contribute to the enhancement of future research by improving the selectivity, sensitivity, and specificity of methods for quantifying tadalafil and losartan potassium together, covering various matrices like fixed-dose combinations, pharmaceutical products, and biological fluids..

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