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Comparative Analysis of Analytical Techniques for Quantification of Valsartan and Sacubitril in Pharmaceutical Preparations: (A-Review)

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ABSTRACT

Accurate measurement of Valsartan and Sacubitril in pharmaceutical formulations is crucial for ensuring both therapeutic efficacy and safety in heart failure treatment. This review compares three analytical techniques-Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), UV-spectrophotometry, and LC-MS/MS-aiming to identify the most effective method for drug quantification. RP-HPLC emerges as the most suitable method for routine analysis due to its superior resolution, precision, and reproducibility. In contrast, UV-spectrophotometry serves as a simpler, cost-effective solution for bulk drug testing in large batches, offering reduced complexity but with less sensitivity. Advanced techniques such as LC-MS/MS and spectrofluorimetric analysis deliver exceptional sensitivity, making them ideal for pharmacokinetic studies; however, they are less practical for everyday testing. Furthermore, green chemistry initiatives are gaining traction as they minimize environmental impact while maintaining high analytical accuracy. Although RP-HPLC remains the preferred method, the evolving landscape of analytical techniques, coupled with sustainable practices, is reshaping the future of pharmaceutical quality control.

Keywords: Valsartan, Sacubitril, RP-HPLC, UV-Spectrophotometry, Green Chemistry.

INTRODUCTION

The LBQ657 prodrug metabolism produces this compound which functions as a neprilysin inhibitor when released into the body[1]. One of the neutral endopeptidases known as neprilysin functions as an endopeptidase that destroys atrial, brain, and C type natriuretic peptides. Stress-based activation of ventricular and atrial receptors increases body levels of ANP and BNP peptides while triggering both fluid loss through diuresis and natriuresis and arterial dilation. Through enzymatic activity neprilysin breaks down various vasoconstrictors including endothelin-1 alongside amyloid beta-protein as well as angiotensin I along with II and multiple vasodilator peptides[2]. When neprilysin activity is blocked normal natriuretic peptides are increased and peptides experience reduced breakdown while also resulting in elevated angiotensin II hormonal levels. Higher natriuretic peptide levels appear when membrane-bound guanylyl cyclase-coupled receptors become active. This sequence of reactions produces renal

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blood circulation improvements with increased diuresis and natriuresis and lowered vascular resistance and greater glomerular filtration. Sacubitril performs enzyme antagonist actions to carry out its pharmacological effects against neprilysin[3].

The white crystalline material known as Valsartan maintains chemical structure $C_{2}4H_{29}N_{5}O_{3}$. This compound shows no water dissolution while it dissolves in alcoholic solutions with pH level controlled solubility occurring between[5–8]. As one of the angiotensin II receptor blockers (ARBs) Valsartan operates together with losartan and telmisartan. At angiotensin receptor 1 (AT_1) Valsartan demonstrates high affinity to bind and prevent angiotensin II from releasing hypertensive reactions[4]. Heart activation combined with blood vessel tightening and elevated aldosterone production and antidiuretic hormone synthesis and sodium retention in the kidneys results from taking Valsartan. The drug Valsartan both regulates the renin-angiotensin-aldosterone system and combines its therapeutic impact on heart functions and blood vessels and kidneys[5]. Through patient administration valsartan releases sodium while lowering blood pressure while also decreasing aldosterone production and heart workload[6].

Objectives

The objective of this review is to critically evaluate and compare the analytical methods used for the quantification of Valsartan and Sacubitril in pharmaceutical formulations. Specifically, the review aims to:

- Assess the performance of three primary analytical techniques-Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), UV-spectrophotometry, and LC-MS/MS-based on their accuracy, sensitivity, precision, and applicability in drug quantification.
- Explore the advantages and limitations of each method in terms of routine analysis, bulk drug testing, pharmacokinetic studies, and stability testing, with an emphasis on their suitability for pharmaceutical quality control[7].
- Investigate the role of emerging green chemistry approaches in enhancing the sustainability of analytical practices, focusing on minimizing environmental impact while maintaining analytical performance and accuracy.

Drug profile

Table 1: Drug profile of Sacubitril

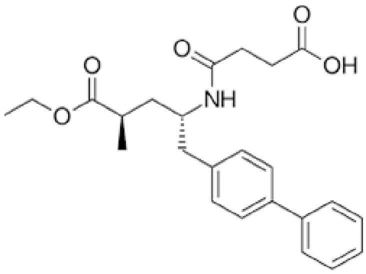
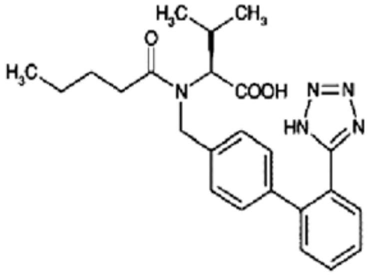
Contents	Sacubitril
Structure	
IUPACname	4-[[(2S,4R)-5-ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl) pentan-2-yl]amino]-4-Oxo butanoic acid.
Description	White powder madeupoftiny hexagonshaped plates
Molecular Formula	C24H29NO5
Molecular Weight	411.49g/mol
PKa	4.6
Melting points	136-138°C
Solubility	Itsiseasily soluble water
Absorption	The maximum drug concentration of Sacubitril occurs at 0.5 hours and LBQ657 at 2 hours. The drug. Sacubitril reaches the market with an absorption rate of 60% through oral administration.
Metabolism	Sacubitril is broken down by esterases into LBQ657.Plasma had a hydroxyl metabolite at a concentration of less than 10%.
Half–Life	Sacubitril exists in human tissue for 1.1 to 3.6 hours yet its breakdown product LBQ657 stays in the body for 9.9 to 11.1 hours.
Excretion	Urine excretion of sacubitril (mostly as the activemetaboliteLBQ657)ranges from 52% to 68%. Fecal excretion of sacubitril (mostly as LBQ657) ranges from 37% to 48%.

Table 2: Drug Profile of Valsartan

Contents	Valsartan
Structure	
IUPACname	(2S)-3-methyl-2-[pentanoyl-[[4-[2-(2(2H-tetrazole-5yl)phenyl]phenyl]methyl]amino]butanoicacid
Description	White crystalline powder
Molecular Formula	$C_{24}H_{29}N_5O_3$.
Molecular Weight	435.52g/mol
PKa	Pka:4.73
Meltingpoint	116-117°C
Solubility	Nature maintains that ethyl methacrylate dissolves almost completely in water while it dissolves entirely in methanol and ethanol (99.5%)
Absorption	Valsartan's antihypertensive effects typically begin within two hours of oral administration, reaching their peak after four to six hours. Food intake reduces peak plasma levels by approximately 50%, while oral administration lowers them by around 40%. With repeated dosages, valsartan does not accumulate significantly in plasma, though its area under the curve increases with dosage
Metabolism	Valsartan undergoes minimal hepatic metabolism, with its primary metabolite, valeryl-4-hydroxyvalsartan, constituting approximately 9% of the administered dose. It is metabolized by the CYP2C9 isoenzyme *in vitro*. Notably, valsartan does not inhibit CYP450 isozymes at clinically relevant doses, reducing the likelihood of drug interactions involving CYP450 enzymes
Half-Life	Valsartan exhibits bi-exponential decay kinetics after intravenous (IV) injection and has an average elimination half-life of six hours
Excretion	Most of the Valsartan substance leaves the body through feces (about 83%) while only 13% reaches urine as an excretion pathway. Most of the medication remains unchanged after receiving the dose yet metabolites appear in 20% of the sample

Mechanisms of action

Entresto, a combination of valsartan and sacubitril, offers a dual approach to treating heart failure. Valsartan inhibits the AT1 receptor, blocking the effects of angiotensin II, while sacubitril's active metabolite, LBQ657, inhibits neprilysin, increasing levels of natriuretic peptides[8]. This combined action provides enhanced protection for both the heart and kidneys. The elevated natriuretic peptides activate membrane-bound guanylyl cyclase-coupled receptors, boosting natriuresis, diuresis, glomerular filtration rate, and renal blood flow. BNP, a biomarker for heart failure, helps guide treatment decisions9. Sacubitril prevents neprilysin from breaking down BNP, promoting vasodilation, sodium excretion, and cardiac remodeling. This leads to improved blood vessel function and heart shape modification. Moreover, sacubitril helps stabilize blood pressure since it affects the metabolism of the angiotensin and other elements useful in managing heart failure and hypertension. It has been observed that the use of both valsartan and sacubitril has a beneficial treatment paradigm in heart failure[10].

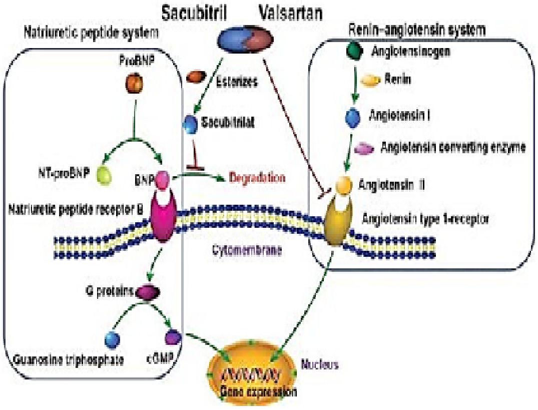


Fig. 1. Mechanisms of Action of Sacubitril valsartan

Chromatographic Method

RP-HPLC Method Development and Validation

The developed RP-HPLC method is suitable for the determination of valsartan and sacubitril in pharmaceutical preparations. By adopting the C18 non-polar stationary phase and employing a polar solvents mixture and organic solvents such as acetonitrile or methanol, this method accedes to some extents of mixtures complexity[11]. The method was used at the epitome of UV absorbance ranges between 230 and 239 nm and isolated peaks with an acetonitrile-buffer ratio of 60:40 and a flow rate of 1.0 mL/minute. These data are supported by validation experiments: linearity was 0.999 or higher, the level of precision was RSD 1,87%, and accuracy ranged from 98,3 to 102%. Built in compliance with ICH Q2(R1) guideline, RP-HPLC can be used as a suitable analytical method to control the quality of valsartan and sacubitril dosage forms[12].

Stability-Indicating RP-HPLC

As far as teh current study is concerned, stability-indicating RP-HPLC method is significant to predict the degradation pattern of valsartan and sacubitril under stressing conditions. It mimics storage and distribution conditions, whereby the drug is exposed to conditions such as acid and base hydrolysis, oxidation, photo degradation and thermal degradation[13]. By employment of C18 column and selective mobile phase, the specific drugs are separated from the dreg products and hence quantified accurately. It is a UV detector at a wavelength of 230 nm, which tracks both the drug and the degradation by-products. This technique abides by the ICH guidelines hence offering a linear, reliable and accurate method of storing data for stability studies which are crucial in estimating shelf life hence facilitates safety in the usage of drugs and medicines[15].

Simultaneous Estimation in Tablet Form

Hence, determination of the concentration of both the active pharmaceutical ingredients, valsartan and sacubitril in their combination tablet dosage forms is very important in pharmaceutical preparations. These drugs can easily be analyzed by reverse-phase high performance liquid chromatography which offers high resolution and sensitivity. This way, RP-HPLC enables the separation of the drugs and excipients on a C18 stationary phase column using mobile phases such as acetonitrile-phosphate buffer or methanol[17]. At a wavelength of 230 nm, both the drugs can be detected and optimization conditions can be controlled during method development such as temperature of the column and the pH of the mobile phase. HPLC Calibration curves exhibit linearity, accuracy and precision according to the ICH guidelines and help in dosage verification and formulation stability.

Spectroscopic Methods

UV-Spectrophotometric Method

UV-spectrophotometry is a simple, precise, accurate, and economical technique for the determination of valsartan and sacubitril in the dosage form. The absorbance of the compounds is done at the wavelengths of 200-400 nm with the compound specific wavelengths of 239 nm for both drugs. The method is based on Beer's Law whereby absorbance is directly associated with the concentration of the analyte in the solution. Thus, calibration curves enable quantification of a drug using the standard solutions According to Dahlberg and screening assays[18]. A two wavelength UV method records spectra at one wavelength that gives highly absorbent value and another that gives a negligible value due to the presence of an excipient. This method is fast and comparatively less expensive than chromatographic methods, and can meet the regulatory requirements in terms of specificity, precision and accuracy for same drug testing.

Multivariate Spectrophotometric Analysis

Multivariate spectrophotometric analysis joins spectrophotometer and statistic analysis to identify one or more than one chemical compound in the sample like Valsartan and Sacubitril in pharmaceutical formulation. It overcomes spectral interferences without the need for additional separation processes. This is done through Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression where raw spectral data is preprocessed in order to obtain the partial components that exhibit maximum variance while mathematical models are built to quantify spectra to drug concentration. The proposed method is free from matrix interferences and no spectral overlap which makes it appropriate for determination of two drugs at once. It includes advantages like sample preparation ease, time saving, and less solvent consumption to provide accurate and consistent analytical testing of quality control for valsartan and sacubitril in tablets as per the regulatory guidelines[20].

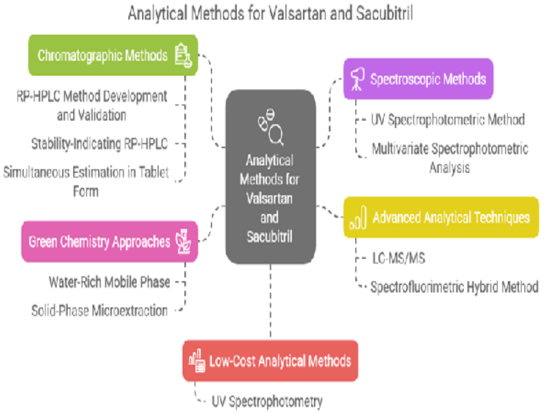


Fig. 2. Analytical methods for Valsartan and Sacubitril

Advanced Analytical Techniques

LC-MS/MS

LC-MS/MS is well applied to determine the concentrations of valsartan and sacubitril in biological matrices and drug formulations at AS Aptitude. This method combining the features of both liquid chromatography where separation is achieved and mass spectrometry where sensitivity and accuracy is the outstanding attraction; makes it possible to detect the presence of the drugs even in plasma samples at very low concentrations. It is done on C18 reverse-phase column using acetonitrile and aqueous buffer solutions as the mobile phase, thereby helping to separate the components, valsartan and sacubitril from other interferences. MS/MS performs a second mass analysis of the initial ions to measure their m/z ratios more accurately. It cuts off the interference by the matrix; enables accurate measurement with background interferences; measures the concentration of the analyte at the range of ng/mL to pg/mL. Compared to other techniques such as HPLC, LC-MS/MS is expensive and highly sophisticated both in terms of equipment and expertise needed in sample preparation; nonetheless, it is vital to pharmacokinetic and most other bioanalytical studies.

Spectrofluorimetric Hybrid Method

A proposed technique involving characteristics of both spectrophotometry and fluorescence spectroscopy was performed to improve the detection and determination of valsartan and sacubitril in pharmaceutical products[21]. This system employs given UV excitation frequencies that in turn, determines the consequent fluorescence; this way, it well detects low drug concentration. This technique is useful for drugs that are themselves naturally fluorescent or can be tagged for fluorescence through derivatization. It also presents an enhanced sensitivity and specificity than any other absorbance techniques with less interferences from the excipients present in formulations. The method has the advantage of speedy analysis and does not require any sample preparation Other than that, the method works best for drugs that are fluorescent or drugs with fluorescence enhancement[22].

Green Chemistry Approaches

Green chemistry analytical techniques refer to methods that try to minimize the use of hazardous solvents and reagents necessary in analyzing samples while at the same time optimizing the efficiency of the analyzed samples and turnaround time to produce reportable data. A accurate and efficient approach for the determination of valsartan and sacubitril compatible with green chemistry guideline by employing water-rich mobile phase with safer co-solvent of ethanol or isopropanol instead of toxic solvent methanol and acetonitrile. It also reduces both hazardous waste and solvent compare to the conventional HPLC as well as UV Visible spectrophotometry. The application of such techniques as solid-phase microextraction (SPME) micro-extraction and solvent-less techniques optimizes the use of solvents to very low levels. These innovations make it possible to get accurate results and apply efficient results for pharmaceutical industries while at the same time keeping the environment a bit greener[23].

Low-Cost Analytical Methods

UV-spectrophotometry is one of the advanced methods that can be effectively used and at the same time economical for analysis of valsartan and sacubitril in pharmaceutical dosage form. This technique is easy, relatively inexpensive and easy to perform compared to other better methods like HPLC or LC-MS/MS. It does not involve the use of many columns and reagents and therefore does not cost a lot of money to set up for use in a laboratory[24]. The method of UV-spectrophotometry analysis is easy to prepare samples and gives rapid results; therefore it is useful in assessing quality control. The method employs methanol or buffer solutions, which are cheaper than those used in chromatographic procedures. Despite having a lesser sensitivity and selectivity compared to other modern techniques, this method of UV-spectrophotometry is affordable, accurate and effective when it comes to the analysis of many drugs and tabletes[25].

Table 3: Comparative Overview of Analytical Methods for Estimation of Valsartan and Sacubitril

Method	Technique Type	Key Characteristics	Advantages	Limitations	Applications
RP-HPLC (Reverse Phase High-Performance Liquid Chromatography)	Chromatographic	High resolution for complex mixtures. Commonly used for routine analysis. Mobile phase composition: acetonitrile and buffer	High accuracy and precision. Suitable for both API and dosage forms	Long analysis time. Need for expensive equipment	Routine analysis of Valsartan/Sacubitril in tablets and injections. Stability testing
UV-Spectrophotometry	Spectroscopic	Fast and cost-effective. Measures absorbance at specific wavelengths (e.g., 239 nm for Sacubitril)	Simple and inexpensive. Easy to set up and operate	Limited sensitivity. Interference from excipients	Quantification of Sacubitril and Valsartan in bulk and dosage forms
HPLC-MS/MS (Liquid Chromatography -Mass Spectrometry)	Chromatographic with Mass Spectrometry	High sensitivity. Useful for trace level analysis. Suitable for complex samples	Extremely sensitive. Ideal for bioanalytical applications (e.g., plasma)	Requires expensive equipment. Complex sample preparation	Bioanalysis (e.g., plasma levels) Pharmacokinetic studies
Spectrofluorimetry	Fluorescence Spectroscopy	Sensitive for low concentration analytes. Uses emission spectrum of the drugs	High selectivity. Can be used for trace analysis	Limited to substances with natural fluorescence	Determination in pharmaceutical formulations. Trace analysis
Ratio Spectroscopy (RS)	Spectroscopic	Use of ratio spectra for overlapping. compounds Can analyze mixtures with complex spectra	No need for separation of. Fast analysis	Less common. components Requires expertise in interpretation	Simultaneous quantification in complex formulations
Stability-Indicating HPLC	Chromatographic	Identifies degradation products. Used under stress testing conditions (e.g., acidic, oxidative)	Ensures regulatory compliance Reliable for stability studies	Requires long validation procedures	Stability testing under various conditions (e.g., light, heat)
Multivariate Analysis (PCR, PLS)	Chemometric	Uses principal component regression (PCR) or Partial least squares (PLS) for complex data analysis	Handles overlapping spectra effectively. Increases sensitivity	Complex data interpretation - Need for software tools	Quantification in complex pharmaceutical formulations
Green Chemistry Approaches	Chromatographic/ Spectroscopic	Eco-friendly solvents used. Reduces environmental impact	- Sustainable and eco-friendly - Reduces hazardous waste	May have limited resolution. Requires optimization	Green analytical methods for routine pharmaceutical analysis
Forced Degradation HPLC	Chromatographic	Studies degradation pathways under forced conditions (e.g., acid, base)	Stability-indicating	More complex method validation Identifies degradation products	Stability studies. Longer run time Forced degradation testing
Hybrid Spectrofluorimetry	Fluorescence Spectroscopy	Combines spectrophotometry and fluorimetry for enhanced sensitivity	High sensitivity for low concentrations. Good for complex matrices	Requires specific expertise. Limited to specific applications	Simultaneous analysis of Sacubitril and Valsartan in complex matrices

Literature Review on Method Development and Validation

Tiris *et al.,* 2024: Spectrophotometric methods and HPLC were used to investigate and estimate SAC and VAL contents in the current study. The HPLC method employed the use of a Waters Spherisorb® column, the mobile phase of acetonitrile and trifluoroacetic acid at a flow rate of 2.0 mL/min and detected at 254.0 nm. Linearity studies showed good linearity, with regression coefficients R^2 equals to 1.00 for both SAC and VAL in the concentration range of 20.0–145.0 µg/mL and 20.0–155.0 µg/mL, respectively. Recovery trials showed low relative standard deviation (RSD) values, confirming method precision. The spectrophotometric method exhibited high linearity ($R^2 = 0.9998$ for SAC and 0.9999 for VAL). This green chemistry-compatible approach ensures cost-effective, rapid, and reliable analysis[26]

Mahendrakumar *et al.,* 2024: In July 2015, the FDA approved the combination of sacubitril (SAC) and valsartan (VAL) for heart failure treatment. A reliable reverse-phase HPLC method was developed for selective separation of SAC, VAL, and their degradation products (DPs). The analysis utilized a Zorbax SB-C8 column with a mobile phase of acetonitrile and ammonium acetate buffer (pH 3.0, 0.02 M) in a 55:45 v/v ratio, with PDA detection at 254 nm and a flow rate of 1.0 mL/minute. Forced degradation studies showed that SAC and VAL exhibited stability under certain conditions, with six degradation products identified. LC-MS and NMR confirmed their structures, demonstrating the method's stability and adherence to ICH Q2 (R1) guidelines for routine analysis[27].

Cholleti, *et al.,* 2023: A stability-indicating RP-HPLC method was developed and validated to detect and quantify five impurities in sacubitril-valsartan tablets. The method employed a Chiralcel OJ-RH column at 45°C with gradient elution at 0.8 mL/min, using a mobile phase of water with trifluoroacetic acid and a mix of acetonitrile and methanol. UV absorbance was observed at 254 nm and LC-MS identified two new degradant formed under base degradation condition. The linearity of the method ranged between 0.999 while the recovery rates were 93 to 105 percent and RSD was below 5.2 percent. This approach is in close compliance with the ICH guideline with respect to the identification of impurities in the originating sacubitril-valsartan drug formulations[28].

Subramanian, Velusamy B., *et al.,* 2022: The determination method of SCB and VLS in tablets was performed following their regulatory methods by applying the design of experiments (DoE) to estimate method robustness. RP-HPLC system was used with a Gemini-NX C18 column (150 x 4.6 mm, 3 µm) and a photodiode array detector was employed in the current study. Gradient mobile phase analysis was carried were at a flow rate of 1.5 mL/min and the column temperature was at 30°C. ICH Q2 (R1) was used to validate this method, and in terms of linearity: R^2 was > 0.999 for all the analytes. Impurity recovery rates ranged from 90.0% to 115.0%, with RSD values below 10.0% for both within-day and between-day measurements. This DoE-based method is capable of effectively quantifying SCB, VLS, and related impurities in drug products, meeting ICH validation standards[29].

Sivaram, Bhadri, *et al.,* 2022: A reverse-phase RP-HPLC method was developed to detect sacubitril impurities in sacubitril-valsartan microsphere formulations, a treatment for heart failure. Given the lack of existing methodologies, researchers employed a Water-X Bridge C18 column (250 mm x 4.6 mm, 5 µm) with gradient elution to separate sacubitril from valsartan in microspheres. A UV detector at 278 nm was used to monitor the mobile phase containing acetonitrile and 10 mM disodium hydrogen phosphate buffer, pumped at 1 mL/minute. Method validation followed ICH Q2 (R1) and Q3B (R^2) guidelines, including system suitability, precision, accuracy, robustness, and forced degradation studies. Sacubitril showed degradation under oxidative, acidic, and UV stress, with a mass balance of 99.5% in stressed samples, confirming the method's stability-indicating capability. The method effectively detects sacubitril impurities in microsphere dosage forms[30].

Kumar, Tatapudi Hemant *et al.,* 2021: A simple, rapid, and accurate RP-HPLC method was developed to simultaneously quantify sacubitril and valsartan. The analysis was performed using an Enable C18 G column in isocratic mode with a mobile phase of methanol and water (60:40 v/v). A UV detector set at 245 nm was used to monitor drug peaks. The method demonstrated a linear relationship between sacubitril and valsartan concentrations ranging from 50 to 450 μg/mL. Method validation followed ICH guidelines, ensuring suitability, accuracy, precision, and robustness. Thus, the method was free from interference from other excipients and could be used for determination of sacubitril and valsartan content in API and tablets[31].

Youssef, Rasha M., *et al.,* 2021: A new simple approach based on spectrofluorimetry has been proposed for determination of VAL and SAC in their binary mixture without any sample separation step. This method combines spectrofluorimetric determination with derivative ratio technique for accurate estimation of both the drugs at the same time. First derivative ratio synchronous fluorescence spectra showed that fluorescence peaks for VAL were at 258-295 nm while for SAC at 204 nm. The inter-day accuracy, precision, recovery, and specificity of the method were established according to ICH guideline, covering a range of 20–200 ng/mL for SAC and 60–200 ng/mL for VAL. The method was proved to be specific, sensitive, and cost-efficient enough to be used in a large-scale routine analysis of LCZ696 tablets and other theraputic combinations.[32]

Bommi, Sivaganesh, *et al.,* 2020: In 2015, the FDA has provided priority nod to the neprilysin inhibitor, SBT known by the brand name Entresto. Stability indicating method was developed according to the QBD approach that is applied to separate 11 impurities. The Ascentis Phenyl Hexyl column, with mobile phase A containing 10 mM KH_2PO_4 buffer at pH 2.1 and mobile phase B consisting of methanol-acetonitrile (70:30 v/v), was employed in gradient mode. The flow rate was set at 0.8 mL/min, with detection at 254 nm. The method demonstrated excellent linearity (r > 0.9989), precision (RSD = 0.9% at 8.0 ppm), and accuracy (92–116%). Impurity quantification limits were set at 0.05%. Forced degradation testing confirmed the method's stability-indicating capabilities[33].

Prajapati, *et al.,* 2020: The SAC/VAL combination therapy is used to manage heart failure, and its analysis can be efficiently performed using ultra-high-performance liquid chromatography (UHPLC), even in the presence of impurities and degradation products. An Accucore XL C8 column (100 × 4.6 mm, 3 μm) at 30°C was used for separation. The gradient elution involved mobile phase A (tetrahydrofuran and 0.1% perchloric acid in water, 8:92 v/v) and mobile phase B (tetrahydrofuran, water, and acetonitrile, 5:15:80 v/v/v). A photodiode array detector analyzed the analytes over 200–400 nm during a 21-min run at 0.6 mL/min flow rate. The method met ICH Q2 (R1) guidelines and demonstrated stability-indicating capability, successfully separating SAC/VAL and related substances under stressed conditions. This UHPLC method is reliable for bulk drug and pharmaceutical product analysis[34].

Moussa, et al., 2018: This study developed and validated an HPLC method for simultaneous detection of sacubitril and valsartan in tablet formulations using C18 stationary phases and UV detection. The mobile phase consisted of 55% acetonitrile and 45% 10^{-3} M cetrimide solution as the ion-pairing agent. Method validation included tests for linearity, precision, accuracy, robustness, and specificity. The method demonstrated excellent accuracy, with recovery rates between 95.0% and 105.0%. Precision was confirmed with RSD values below 5.0%, and high specificity was maintained in the presence of degradation products. This RP-HPLC method is effective for routine pharmaceutical quality control, offering simple operation and reliable performance[35]

Madhuri, P. Leela *et al.,* 2019: Measurement of sacubitril contents in synthetic mixtures can be done by UV-spectrophotometry. This method is precise, sensitive, and specific. A thorough validation of linearity, accuracy, precision, sensitivity, and measurement range was achieved in compliance with ICH guidelines. Under visible light an UV absorption spectrum maximum appeared at 242 nm when merge sacubitril with a methanol-water solution (25:75 V/V). The presented linearity tests worked well at concentrations from 2 to 12 μg/mL while simultaneously producing an r-value of 0.999. The validation tests proved that the method met ICH requirements which proved its reliability as a method. A proposed test methodology demonstrates high sensitivity, simplicity and affordable operation while constituting an appropriate method for pharmaceutical formulation sacubitril analysis[36].

Khder, *et al.,* 2017: This study developed a combined HPTLC and HPLC method with fluorescence (FLD) and diode array detectors (DAD) to analyze sacubitril (SAC), valsartan (VAL), and biphenyl methyl pyrrolidinone (BMP), a SAC-related impurity. The goal was to identify and quantify BMP in SAC measurements. HPLC-FLD and HPLC-DAD were used to detect BMP at concentrations of 0.169%, 1%, and 3%. Detection sensitivity was high, with detection limits of 8.3 µg/mL for VAL, 3.3 µg/mL for SAC, and 1.7 µg/mL for BMP. Quantification limits were 25 µg/mL for VAL, 10 µg/mL for SAC, and 5 µg/mL for BMP. This method demonstrated precise detection of BMP trace levels, making it suitable for impurity analysis in SAC and LCZ696 formulations.[37]

Amoss, et al., 2011: This study developed a successful HPLC method for detecting and quantifying sacubitril (SA) and valsartan (VA) using stereo-selective normal-phase chromatography. Chiralcel OJ-H columns (250 mm x 4.6 mm) with n-hexane and ethanol as mobile phases enabled efficient stereoisomer separation at 1.0 mL/min and 254 nm over 50 minutes. The method showed high accuracy (98.3–99.5%), precision (RSD<1.82%), and linearity ($R^2>0.998$). It also demonstrated low detection (0.06 µg/mL) and quantification (0.2 µg/mL) limits, providing precise analysis of stereoisomeric impurities in drug formulations[38].

Raul, *et al.,* 2016: Two UV-spectrophotometric methods were developed to accurately and precisely analyze valsartan in raw materials and tablets. Method I, using a zero-order spectrum, showed a maximum absorbance at 250 nm, while Method II, utilizing the second-order derivative spectrum, had a λmax at 241 nm. Both methods demonstrated excellent linearity ($r^2 = 0.999$) across the 10-50 µg/mL range and were validated for robustness, ruggedness, and specificity, making them suitable for routine quality control in pharmaceutical formulations[39].

CONCLUSION

In accurate estimation of valsartan and sacubitril in pharmaceutical formulations is critical for ensuring the safety and efficacy of heart failure treatments. Consequently, analytical methods such as RP-HPLC, UV-spectrophotometry, LC-MS/MS, and hybrid spectrofluorimetric methods have their own pros and cons based upon the context of their use. It is an essential analytical technique that cannot be replaced by any other approach because it is perceptive, concise, and capacious for sophisticated samples. It is suitable for both primary sample analysis of an active pharmaceutical ingredient (API) as well as stability examination. However, UV-spectrophotometry is cheaper and more straightforward as a method for the determination of concentration of bulk drugs even though it is less sensitive compared to chromatographic methods. LC–MS/MS is especially sensitive and quite useful for pharmacokinetic studies and for detecting trace levels; however, it cannot efficiently analyze samples with complex matrices, and its cost is quite high. Novel green chemistry strategies that ensure low toxicity of the solvents used and possess low environmental impact whilst providing the same effectiveness of analyses are presented. The pharma QA industry is now featuring a gradual shift towards greener solutions as part of the current pharmacopeial requirements and trends. In the future, all these methods can be integrated and customized according to the necessities of the testing process to allow more efficient, cheap and ecologic pharmaceutical analysis.

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Conflict of interest

Regarding this paper, the authors state that they have no relevant conflict of interest.

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