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HPTLC Approach for Simultaneous Quantification of Valsartan and Sacubitril in Bulk and Tablet Formulations

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Abstract

The article introduces an inventive, eco-friendly, simple, consistent and reproducible NP-TLC-Densitometry approach to simultaneous pharmaceutical estimation of Valsartan and Sacubitril in bulk and tablet matrix. Valsartan and Sacubitril is a combination of two medications used to treat high blood pressure (hypertension). This combination is used when a single drug is not enough to control blood pressure. The determination was performed by employing densitometric estimation using ultraviolet exposure at 252 nm. The separation was achieved on (10 × 10 cm) aluminium backed silica gel 60-F_{254} as stationary phase. Optimized mobile phase was dichloroethane: methanol: triethylamine (4.2:0.4:0.4 v/v/v). Quantitation was conducted over a concentration range of 260 - 1560 ng/band of valsartan and 240 - 1440 ng/band of sacubitril. The compact and well-resolved bands for both drugs in standard and samples were obtained at a retention factor (Rf) value of 0.57 ± 0.02 and 0.42 ± 0.02 for valsartan and sacubitril respectively. As per International Council for Harmonization the established method was successfully validated to various parameters like accuracy, precision, sensitivity, specificity, robustness and shows the satisfactory results for all parameters. The recognized method is simple, accurate, precise, robust, sensitive and economical in nature. This method can be used for quality control of Valsartan and Sacubitril in bulk and in combined dosage form.

Keywords: Valsartan; Sacubitril; HPTLC; ICH

1. Introduction

Valsartan (VLS) (Figure 1) is chemically (S)-3-methyl-2-(N-{[2'-(2H-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl]methyl}pentanamido)butanoic acid. It is an orally active nonpeptide triazole-derived antagonist of angiotensin (AT) II with antihypertensive properties. Valsartan selectively and competitively blocks the binding of angiotensin II to the AT1 subtype receptor in vascular smooth muscle and the adrenal gland, preventing AT II-mediated vasoconstriction, aldosterone synthesis and secretion, and renal reabsorption of sodium, and resulting in vasodilation, increased excretion of sodium and water, a reduction in plasma volume, and a reduction in blood pressure [1, 2].

Sacubitril (SCB) (Figure 2) is chemically 4-{[(2S,4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl]amino}-4-oxobutanoic acid. It is a neprilysin inhibitor used in combination with valsartan as an adjunct to reduce the risk of cardiovascular death and hospitalization for heart failure in patients with chronic heart failure (NYHA Class II-IV) and reduced ejection fraction. Sacubitril is a prodrug that is activated to sacubitrilat (LBQ657) by de-ethylation via esterases.

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Sacubitrilat inhibits the enzyme neprilysin, which is responsible for the degradation of atrial and brain natriuretic peptide, two blood pressure–lowering peptides that work mainly by reducing blood volume [3].

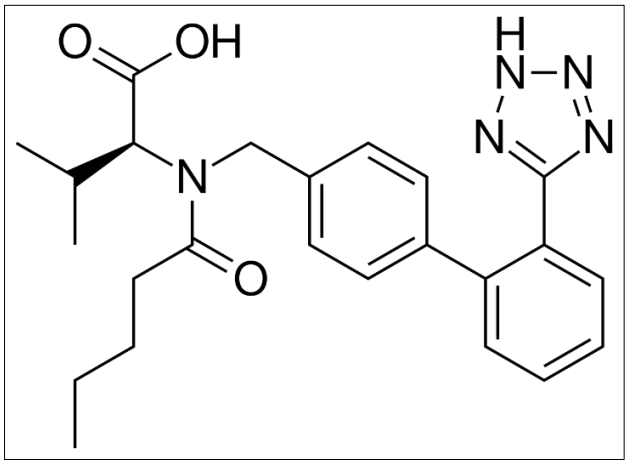

Figure 1 Chemical Structure of Valsartan

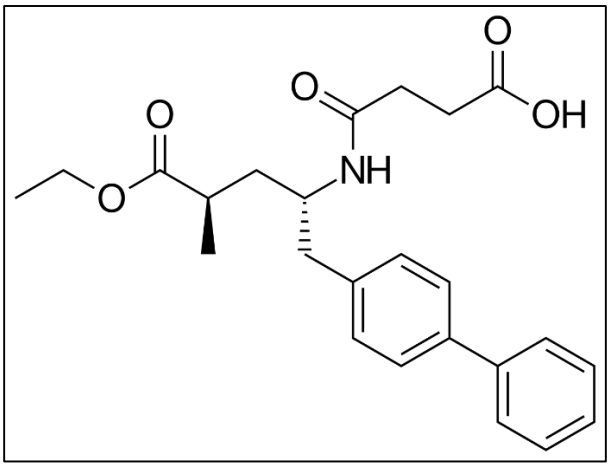

Figure 2 Chemical Structure of Sacubitril

In literature, several methods are reported for the quantification of Valsartan and Sacubitril in comibination or alone from bulk, pharmaceutical formulation, and biological fluids. Analytical methods reported in literature includes UV-spectrophotometry [4-8], RP-HPLC [9-18], spectrofluorimetry [19, 20], HPTLC [21] and LC-MS/MS [22]. The methods that are reported in the literature have several advantages and also limitations. The HPLC approach is more sensitive; however, it necessitates a greater quantity of solvent and a higher proportion of buffers, resulting in extended analytical determination time and potential control issues in the analysis. Moreover, the developed HPTLC approach will necessitate reduced solvent volumes, facilitating cost-effective analysis and enabling the examination of a substantial number of samples in a brief timeframe [23-25].

Therefore, an objective of the present work is to develop and validate HPTLC method for simultaneous determination of Valsartan and Sacubitril in bulk and pharmaceutical formulation using ICH guidelines [26].

2. Experimental method

2.1. Drugs and Reagents

Pharmaceutical grade Valsartan and Sacubitril working standards were a generous gift from Macleods Pharmaceuticals ltd. Mumbai, India. Fixed dose tablet formulation VYMADA® containing 26 mg of VLS and 24 mg of SCB purchased from local pharmacy store. HPLC grade dichloroethane, methanol and triethylamine are used as mobile phase and procured from Merck (India) Ltd., Worli, and Mumbai, India.

2.2. Instrumentation

Camag TLC system (Muttenz, Switzerland) involves of Camag Linomat 5 sample applicator, Hamilton syringe (100 µL), Camag TLC scanner 3, Camag winCATS software (version 1.3.0), Camag twin trough chamber (10 x 10 and 10 x 10 cm) and ultrasonicator; ENERTECH Electronics Pvt. Ltd., India were utilizing throughout the analysis. Normal-phase chromatography separation was performed on 10 cm × 10 cm silica gel F_{254} HPTLC plates having 200 µm thicknesses (E. Merck, Mumbai, India). Before to use, NP-HPTLC plates washed with methanol and dried in oven at 110˚C for 5 mins. The quantification was carried out using TLC scanner 3 (Camag) installed with winCATS software (version 1.3.0), Drug sample applied on HPTLC plates using Linomat 5 applicator (Camag) under nitrogen gas flow. Plate were development in a twin trough chamber, with dichloroethane: methanol: triethylamine (4.2:0.4:0.4 *v/v/v*). as mobile phase for NP-HPTLC respectively, chamber saturation time 20 min at room temp (28˚C ± 2) for both methods. The scanning was done using densitometric TLC scanner 3 equipped with winCATS software version 1.3.0 at 252 nm in absorbance and reflectance mode with deuterium lamp emitting a regular UV-spectrum between 200 nm - 800 nm.

2.3. Preparation of mixed stock standard solution

Mixed stock standard solutions of VLS (260 µg/mL) and SCB (240 µg/mL) was prepared by dissolving 26 mg of VLS and 24 mg of SCB in 100 mL methanol.

2.4. Linearity Studies of VLS and SCB

From the mixed stock standard stock solution, 1- 6 mL was transferred into series of six volumetric flasks and volume was made up to the mark with methanol. From each volumetric flask a volume 10 µL was applied on HPTLC plate to obtain series of concentration 260 - 1560 ng/band of VLS and 240 - 1440 ng/band of SCB. The plates were developed and scanned as described under above established chromatographic conditions. Each standard in six replicates was analyzed and peak areas were recorded. Calibration curves of VLS and SCB were plotted separately of peak area *vs.* respective concentration of VLS and SCB. The results are shown in Table 1 and 2, the calibration curves are shown in Figure 3 and 4.

Table 1 Linearity Study of VLS

Concentration (ng/band)	Area ± SD	%RSD (n=6)
260	5628± 72.04	1.28
520	8033± 82.74	1.03
780	10797 ± 101.49	0.94
1040	13204 ± 116.20	0.88
1300	15425 ± 115.69	0.75
1560	17841 ± 123.10	0.69

Table 2 Linearity Study of SCB

Concentration (ng/band)	Area ± SD	%RSD (n = 6)
240	3990 ± 40.70	1.02
480	5914 ± 48.49	0.82
720	7805 ± 71.03	0.91
960	9596 ± 82.53	0.86
1200	11446 ± 89.28	0.78
1440	12999 ± 106.59	0.82

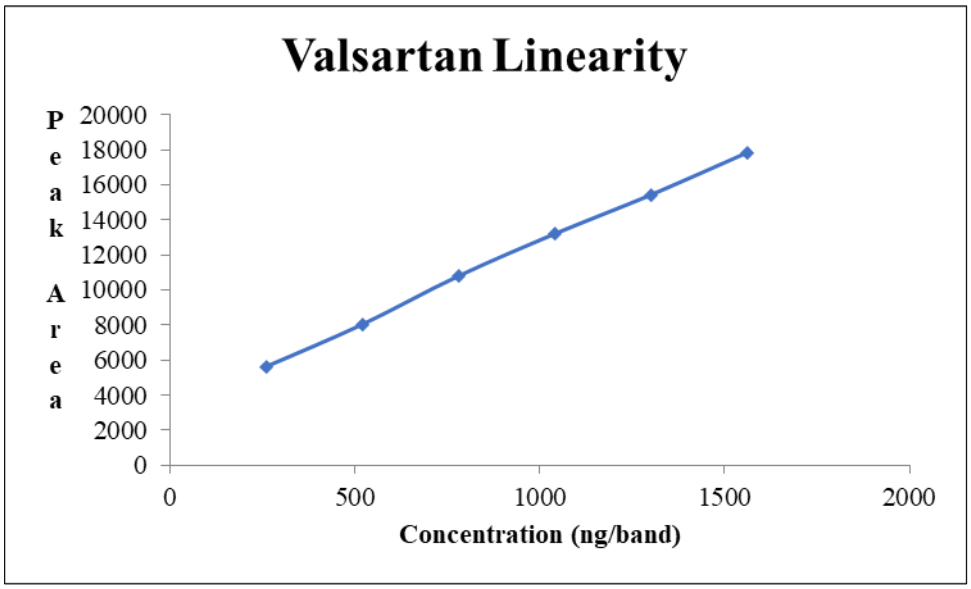

Figure 3 Calibration curve for Valsartan

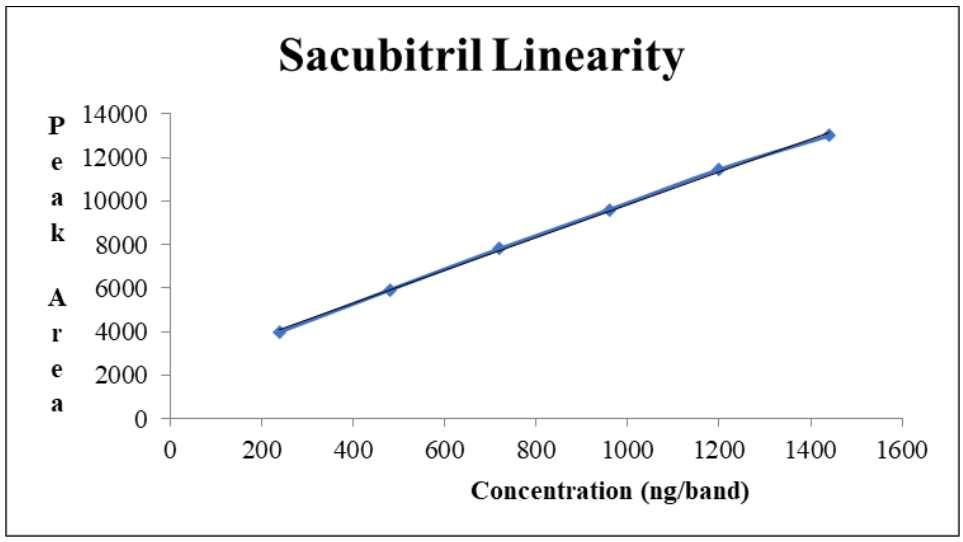

Figure 4 Calibration curve for Sacubitril

2.5. Analysis of bulk material

Accurately weighed 26 mg of VLS and 24 mg of SCB were transferred into 100 mL volumetric flask containing 30 mL of methanol, shaken manually and volume was adjusted to mark using same solvent. 3 mL further diluted to 10 ml with methanol and appropriate volume of 10 µL of this solution containing 780 ng of VLS and 720 ng of SCB was applied on HPTLC plate. The plate was developed, dried and scanned as described above. The concentration was determined by regression equation and results are shown in **Table 3.**

Table 3 Analysis of bulk material

Component	Amount Taken (ng/band)	Amount Found (ng) ± SD	%RSD [n=6]
VLS	780	10557.83 ± 77.25	0.731
SCB	720	7713.66 ± 67.40	0.874

2.6. Analysis of Tablet formulation

Twenty tablets (Vymada) were weighed and finely powdered. An amount of powder equivalent to 26 mg of VLS and 24 mg of SCB was transferred to 100 mL volumetric flask containg 70 ml methanol and sonicated for 10 min. The solution was diluted to volume with the same solvent and filtered through Whatmann No.41. Resulting solution (3 mL) was further diluted to 10 mL with the same solvent. From it, an appropriate volume of 10 µL was applied on TLC plate followed by development and scanning as described as above. The concentration of drugs was determined from linear regression equations and % label claim was calculated, shown in Table 4.

Table 4 Analysis of Tablet Formulation

Brand Name: VYMADA			**Mfg. By: Novartis India Ltd.**	
Component	**Label Claim [mg]**	**Amount Found ± SD [ng]**	**% Label Claim**	**% RSD [n=6]**
VLS	26	10552.83± 64.77	99.40	0.887
SCB	24	7712 ±68.37	99.89	1.258

2.7. Validation

The proposed NP-HPTLC method was validated as per ICH guidelines to secure them for linearity, precision, selectivity, sensitivity, robustness, accuracy and specificity.

2.8. Accuracy

Recovery experiments were performed at three different levels i.e. 80, 100 and 120 %. To the pre-analysed sample solutions, a known amount of mixed drug standard solutions of VLS and SCB were over spotted at three different levels. The chromatogram was developed and scanned as described above; the results of % recovery are shown in **Table 5**

Table 5 Results for Recovery studies

Components	**Initial Amount [ng/band]**	**Amount added (%)**	**Amount recovered ± S.D. [ng/band] [n=3]**	**% Recovered**	**% RSD**
VLS	520	80	934	99.51	1.32
	520	100	1035	99.03	1.12
	520	120	1140	99.35	0.87
SCB	480	80	860	98.95	1.41
	480	100	956.22	99.21	1.23
	480	120	1053.5	99.56	1.05

2.9. Precision (Intra- day and Inter- day precision)

Precision of the method was studied as repeatability and intra-day and inter-day variations.

The repeatability of sample application and measurement of peak area was determined by performing six replicate measurements of the same using 780 ng/band for VLS and 720 ng/band for SCB. Intra-day variation was determined by analyzing three different concentrations for three times within a day and Inter-day precision was assessed by three different concentrations for three different days, over a period of week. The intra-day and inter-day variation was measured at three different concentrations 520, 780, 1040 ng/band (VLS) and 480, 720, 960 ng/band (SCB). The results are shown in Table 6.

Table 6 Results for Precision Studies (Intra- day and Inter- day)

Drugs	Conc. [ng/band]	Intra day Amount found [ng]		Inter dayAmount found [ng]	
		Mean ± SD [n= 3]	% RSD	Mean ± SD [n= 3]	% RSD
VLS	520	8123.23 ± 78.12	0.961	8105.78 ±86.45	1.066
	780	10574.44 ±95.01	0.898	10551.4 ±101.91	0.965
	1040	12984.32 ±66.54	0.512	12936.42 ±99.55	0.769
SCB	480	5884.32 ±72.47	1.231	5865.35±74.33	1.267
	720	7671.74±68.14	0.888	7662.51±71.21	0.929
	960	9495.14±59	0.621	9485.14±62.78	0.661

2.10. Specificity

The mobile phase designed for the method resolved both the drugs very efficiently; Figure 5. The R_F value of VLS and SCB was found to be 0.57 ± 0.02 and 0.42 ± 0.02, respectively. A typical absorption spectrum of VLS and SCB is shown in Figure 6 .The peak purity of VLS was assessed by correlating the spectra of standard VLS and VLS- extracted from tablets at the peak-start (S), peak-apex (A) and at the peak-end (E) positions. Correlation between these spectra indicated purity of VLS peak {correlation *r*(S, M) = 0.998, *r* (M, E) = 0.999}; Figure 7 The peak purity of SCB-extracted from SCB and standard SCB was assessed by correlating the spectra of SCB at the peak-start (S), peak-apex (A) and at the peak-end (E) positions. Correlation between these spectra indicated the purity of SCB peak {correlation *r*(S, M) = 0.998, *r* (M, E) =0.999}; Figure 8 Thus, it can be concluded that no impurities or degradation products were found with the peaks of standard drug solutions.

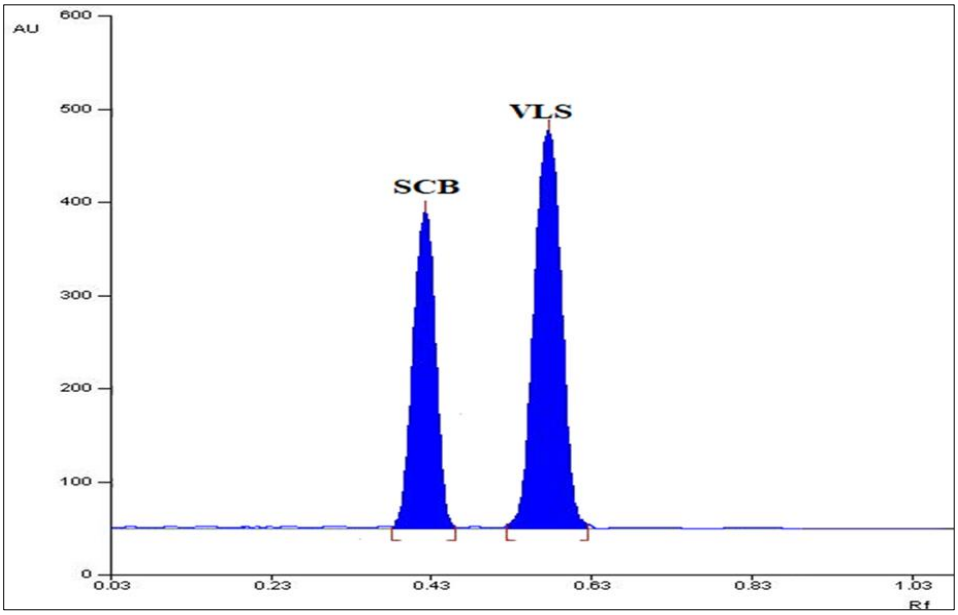

Figure 5 NP-HPTLC Densitogram of VLS (780 ng/band, R_f 0.57 ± 0.02) and SCB (720 ng/band, R_f 0.42 ± 0.02) drug solutions in Dichloroethane: Methanol: triethylamine (4.2:0.4:0.4 *v/v*)

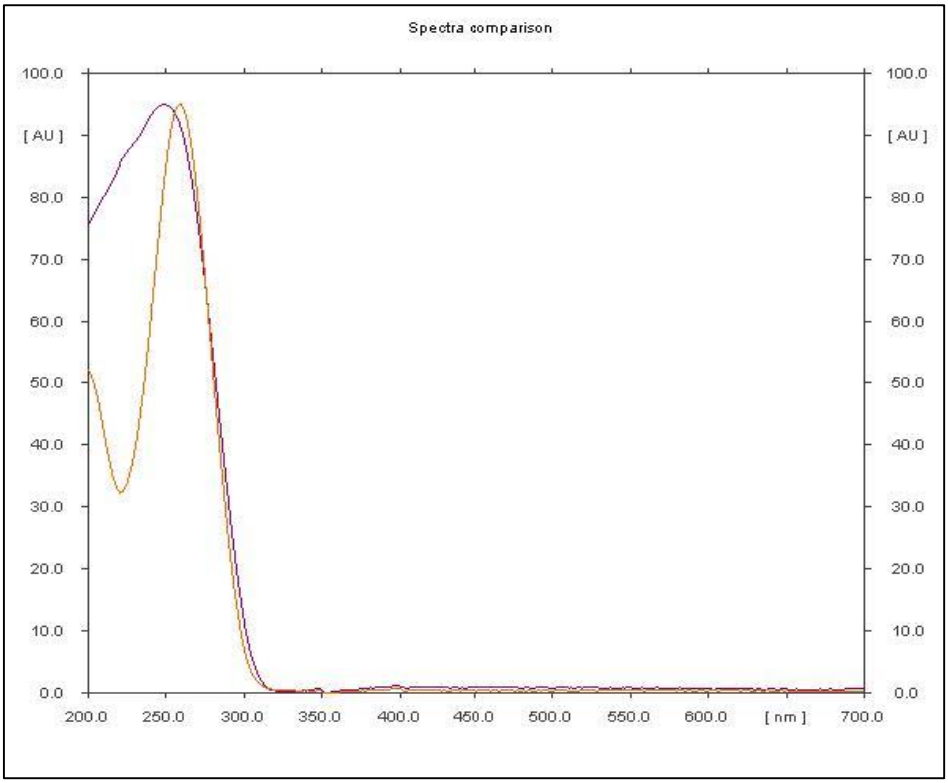

Figure 6 Typical overlay spectra of standard VLS and SCB

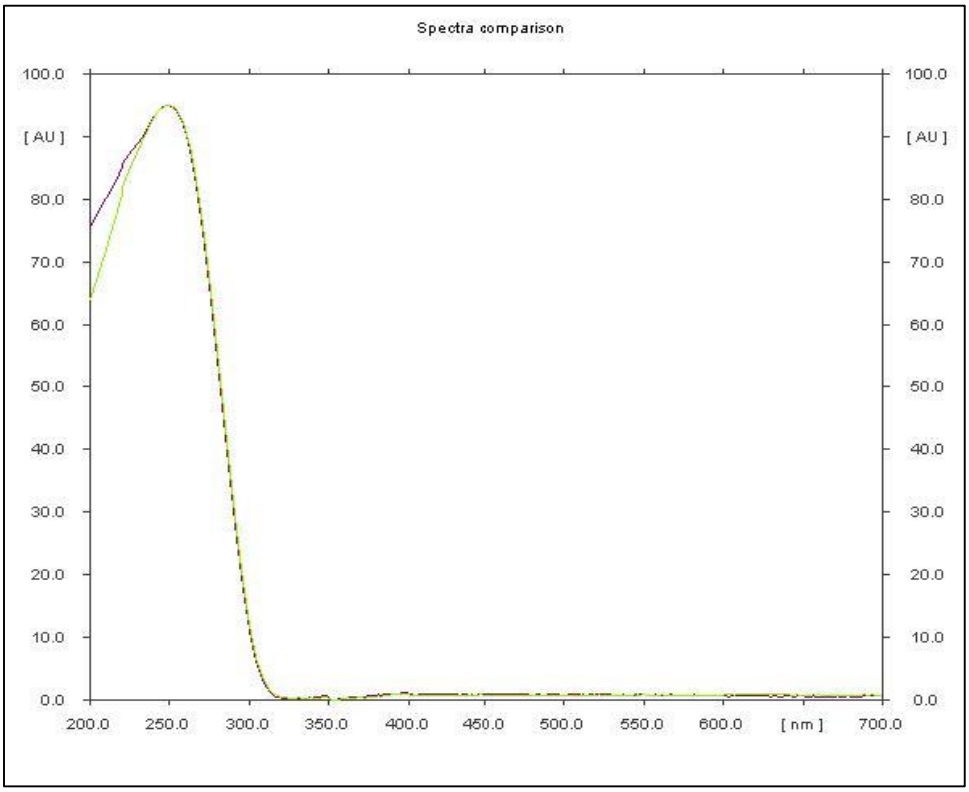

Figure 7 Peak purity spectra of standard 1 VLS, sample 2 extracted from tablet, scanned at the peak-start, peak - apex and peak-end positions of the band (Correlation > 0.99)

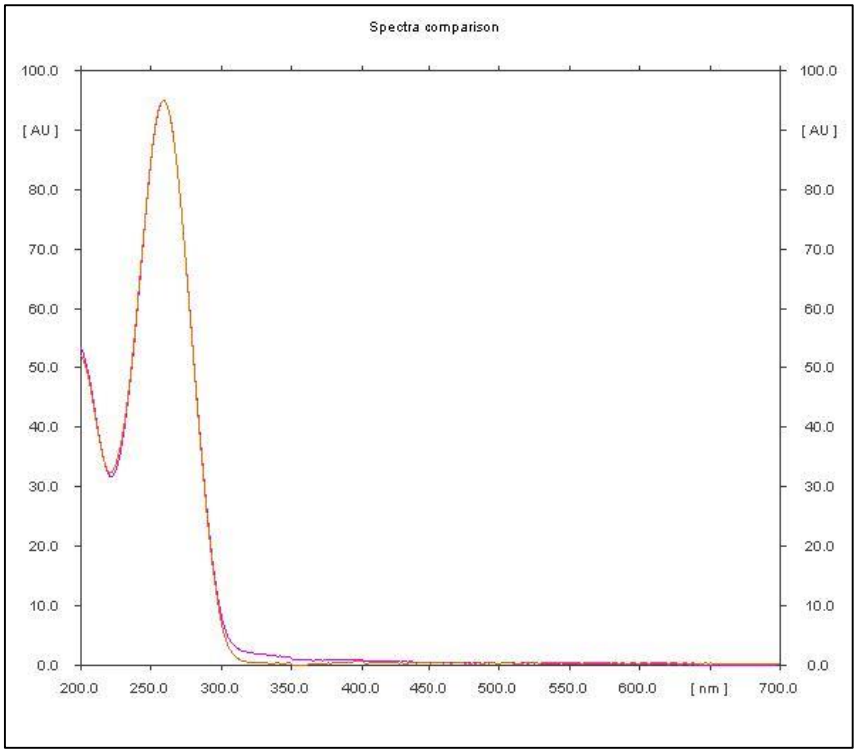

Figure 8 Peak purity spectra of standard 1 SCB, Sample2 extracted from tablet, scanned at the peak - start, peak- apex and peak-end positions of the band(Correlation > 0.99)

2.11. Sensitivity

The sensitivity of measurement of VLS and SCB by the use of the proposed method was determined in terms of the LOD and LOQ. The LOD and LOQ were calculated using equation LOD = 3.3 X N/B and LOQ = 10 X N/B; Where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. Mixed stock solution of VLS and SCB was prepared and different volume in the range 260 - 520 ng/band of VLS and 240 - 480 ng/band of SCB were applied in triplicate. The LOD and LOQ for VLS were found to be **24.84 ng** and **75.30ng**, respectively. For SCB the LOD and LOQ was found to be **15.83 ng** and **47.96 ng**, respectively.

2.12. Ruggedness

Ruggedness of the proposed method was studied by two different analysts using the same experimental and environmental conditions. The band 780 ng/band of VLS and 720 ng/band of SCB were applied on HPTLC plates. The development and scanning of bands were performed as described above. This procedure was repeated in triplicates; the results are integrated in terms of %RSD. The results were found to be within accepted range.

2.13. Robustness

Table 7 Results of Robustness Studies

Parameters	**VLS**		**SCB**	
	± SD of peak area [n = 6]	**% RSD**	**± SD of peak area [n = 6]**	**%RSD**
Mobile phase volume	39.25	1.65	9.31	1.56
Mobile phase composition	41.61	1.75	6.78	1.13
Development distance	34.60	1.46	10.51	1.75
Duration of saturation	16.62	0.70	4.38	0.73

Robustness was studied in six replicate at the concentration 780 ng/band of VLS and 720 ng/band of SCB, respectively. In this experiment, four parameters (mobile phase composition, mobile phase volume, development distance, duration of saturation) were studied; the results are shown in Table 7.

3. Conclusion

A novel, simple, and precise HPTLC (High-Performance Thin-Layer Chromatography) technique has been successfully developed and validated for the determination of valsartan (VLS) and sacubitril (SCB) in bulk and combined tablet dosage forms. This validated method is simple, precise, rugged, and robust, making it highly suitable for the simultaneous estimation of both valsartan and sacubitril. Additionally, the method has demonstrated accuracy and sensitivity, ensuring reliable results. The percent recovery in the formulation indicates that the excipients present do not interfere with the determination of the active pharmaceutical ingredients. This confirms that the proposed method is specific and unaffected by formulation components, making it ideal for routine analysis. The proposed method can be routinely explored for the simultaneous estimation of valsartan and sacubitril in both bulk and tablet dosage forms. The method provides a reliable tool for quality control and analysis in pharmaceutical industries, ensuring consistent results in drug formulations.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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