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Spectrophotometric Quantification of Valsartan in Pure Substance and Pharmaceutical Formulations through Ion-Pair Complexation with Bromophenol Blue and Methyl Red without Extraction

Ryota Nakamura[1]*, Yui Tanaka[1], Sota Suzuki[1]

[1]Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan.

***E-mail** ✉ ryota.nakamura@gmail.com

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ABSTRACT

Two environmentally friendly, quick, and straightforward non-extractive spectrophotometric techniques have been developed for the quantitative estimation of valsartan in tablet formulations. The procedures rely on the formation of ion-pair complexes between valsartan and the acidic dyes bromophenol blue (BPB) and methyl red (MR). The drug interacts selectively with these dyes, generating colored complexes—yellow with BPB at pH 5.5, showing maximum absorbance at 424 nm, and red with MR at pH 4.3, with λmax at 494 nm. Optimal analytical conditions were determined for both methods. A linear correlation was observed between absorbance and drug concentration within the ranges of 8–24 µg/mL for BPB and 4–20 µg/mL for MR. The regression equations were found to be y = 0.0102x + 0.01636 (BPB) and y = 0.0222x – 0.0063 (MR), with excellent correlation coefficients (R^2 = 0.9988 for BPB and R^2 = 0.9991 for MR), confirming adherence to Beer's law. The limits of detection (LOD) and quantification (LOQ) were determined as 1.03 µg/mL and 3.43 µg/mL for BPB, and 0.68 µg/mL and 2.26 µg/mL for MR, respectively. Accuracy, precision (both intra- and inter-day), and robustness studies demonstrated satisfactory results. The validated methods were successfully utilized for the determination of valsartan in three marketed tablet brands. Evaluation using the Analytical Eco-Scale indicated that both approaches represent excellent green analytical procedures, each achieving a score of 89.

Keywords: Valsartan, Bromophenol blue, Analytical Eco-Scale, Spectrophotometry, Methyl red

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Introduction

Valsartan is an antihypertensive medication that functions as a selective angiotensin II receptor blocker (ARB), specifically targeting the AT1 receptor subtype. It is clinically employed in the management of hypertension, post-myocardial infarction conditions, and symptomatic heart failure—particularly in patients who cannot tolerate ACE inhibitors or as an adjunct to ACE inhibitors when β-blockers are contraindicated. Chemically, valsartan is identified as (2S)-3-methyl-2-[pentanoyl[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]amino]butanoic acid **(Figure 1)** [1].

According to the European Pharmacopeia [1], valsartan is officially monographed, with its identification established through infrared absorption spectrophotometry, assessment of enantiomeric purity, and optical rotation. The quantitative determination is carried out by titrimetry using 2-propanol as the solvent and 0.1 M tetrabutylammonium hydroxide as the titrant.

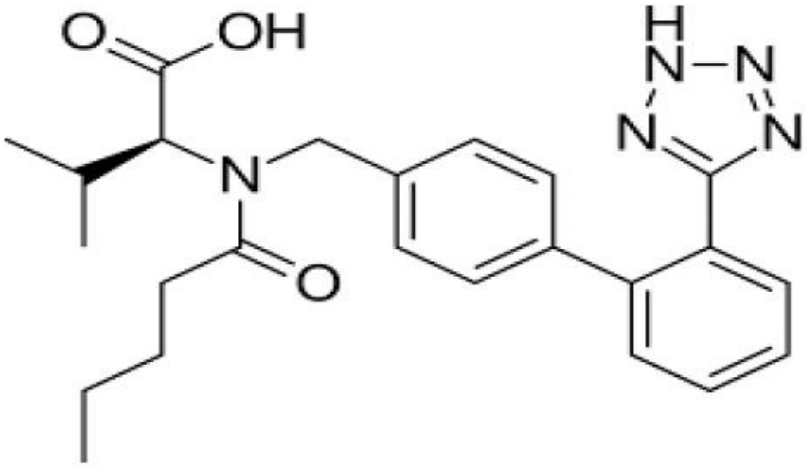


Figure 1. Chemical structure of valsartan.

Several analytical investigations have explored spectrophotometric procedures for the quantification of valsartan, either by measuring its intrinsic absorption spectrum [2-9] or through its chemical reaction with various chromogenic reagents [10, 11]. In one investigation, Indian researchers proposed spectrophotometric methods for the simultaneous estimation of valsartan and ezetimibe in pharmaceutical formulations utilizing sulfophthalein dyes such as bromophenol blue and bromocresol green [10]. Their approach depended on the formation of ionic associates between valsartan and the dyes, producing yellow-colored ion-pair complexes that showed bathochromic shifts with absorption maxima at 425 nm and 428 nm, respectively. The interaction between valsartan and each dye was found to occur in a 1:1 molar ratio.

Similarly, scientists at Minia University in Egypt developed both spectrophotometric and spectrofluorimetric procedures for determining angiotensin II receptor blockers—namely losartan, irbesartan, telmisartan, and valsartan—in raw materials and dosage forms [11]. Their spectrophotometric method involved complex formation between these drugs and sulfophthalein dyes, yielding stable yellow complexes with maximum absorbance within the range of 413–419 nm. In contrast, their spectrofluorimetric method was based on generating non-extractive binary complexes of eosin with the same set of drugs, including valsartan.

Despite their effectiveness, many of these analytical techniques require elaborate preparation, long processing time, or involve environmentally unfriendly reagents, which restricts their practical application in routine analysis. Hence, there remains a demand for a rapid, straightforward, and sustainable spectrophotometric approach for valsartan assay in tablets, especially in contexts where simplicity, accuracy, and low cost are essential.

The present study introduces two fast, eco-friendly, and non-extractive spectrophotometric methods for valsartan estimation in tablet formulations. These techniques are based on ion-pair complex formation with the dyes bromophenol blue (BPB) and methyl red (MR).

Aim of the work

The objective of this research was to design and validate simple, rapid, and environmentally sustainable non-extractive spectrophotometric methods for determining valsartan content in tablet dosage forms.

Materials and Methods

Apparatus

Absorbance measurements were carried out using a Shimadzu UV–Visible double-beam spectrophotometer (Model UV-1800, Japan), equipped with UV-Probe 2.62 software and 1 cm matched quartz cuvettes. The instrument features a 1 nm spectral bandwidth and a wavelength accuracy of ±0.5 nm. Conforming to Japanese and European Pharmacopeia specifications, the UV-1800 provides superior resolution (1 nm) in a compact configuration, making it ideal for high-precision analytical studies.

Reagents and standards

All reagents and solvents used in this study were of analytical grade. The reference standard of valsartan (≥98% purity, HPLC) was obtained from Sigma-Aldrich. Bromophenol blue (BPB) and methyl red (MR) dyes were sourced from Honeywell Fluka and prepared as stock dye solutions — BPB at 1.3×10^{-3} M in methanol, and MR at 3.7×10^{-3} M in ethanol.

Spectrophotometric estimation of Valsartan with Bromophenol Blue (BPB)

Procedure for standard solution

To prepare the working solution, 24.54 mg of valsartan CRS was dissolved in 15 mL of methanol in a 25 mL volumetric flask, shaken thoroughly, and diluted to the mark with methanol. From this, 0.4 mL was mixed with 1.0 mL of BPB solution (1.3×10^{-3} M in methanol) and made up to 25 mL with methanol. The absorbance of the resulting colored complex was recorded at 424 nm against a reagent blank prepared without the drug. A calibration curve was constructed by plotting absorbance versus concentration.

Procedure for tablet formulation

Twenty tablets were weighed, ground, and a sample equivalent to 24.54 mg of valsartan was transferred to a 25 mL volumetric flask containing 15 mL of methanol. The mixture was agitated for 15 minutes, diluted to the mark, and filtered through a 0.2 µm nylon membrane. Then, 0.4 mL of this filtrate was treated with 1.0 mL of 1.3×10^{-3} M BPB solution, and the total volume was adjusted to 25 mL with methanol. Absorbance was recorded at 424 nm against the reagent blank, and the calibration graph was plotted accordingly.

Spectrophotometric estimation of Valsartan with Methyl Red (MR)

Procedure for standard solution

An accurately weighed 11.73 mg of valsartan CRS was dissolved in 15 mL of ethanol in a 25 mL volumetric flask, shaken, and diluted to volume. From this, 0.3 mL was combined with 0.5 mL of MR solution (3.7×10^{-3} M in ethanol), and the mixture was diluted to 25 mL with ethanol. The absorbance was measured at 494 nm relative to a reagent blank without valsartan, and the calibration curve was prepared from the absorbance–concentration relationship.

Procedure for tablet formulation

After weighing and pulverizing twenty tablets, a portion equivalent to 11.73 mg of valsartan was transferred into a 25 mL volumetric flask containing 15 mL ethanol, shaken for 15 minutes, diluted to volume with ethanol, and filtered through a 0.2 µm nylon membrane. Then, 0.3 mL of this solution was mixed with 0.5 mL of MR solution (3.7×10^{-3} M in ethanol), diluted to 25 mL with ethanol, and the absorbance was recorded at 494 nm against the reagent blank. Calibration was achieved by plotting absorbance versus concentration.

Validation of analytical methods

The developed procedures were validated in accordance with ICH guideline Q2 [12]. The validation covered essential analytical parameters such as specificity, linearity, detection and quantification limits, working range, precision (intra- and inter-day), accuracy, and robustness.

Results and Discussion

Method development

The spectrophotometric approach was based on the formation of non-extractive ion-pair complexes between valsartan and the acidic dyes BPB and MR. This interaction is attributed to electron-donating functional groups (such as the tetrazole and amino butanoic acid moieties) in the valsartan molecule, which facilitate ion-pair formation. The complexation produced distinct colored products—yellow with BPB at pH 5.5 and red with MR at pH 4.3—exhibiting absorption maxima at 424 nm and 494 nm, respectively. Although these complexes exhibited limited solubility in water, they dissolved completely under optimized solvent conditions without requiring extraction or surfactant addition. The absorption spectra illustrating these interactions are shown in **Figures 2 and 3**.

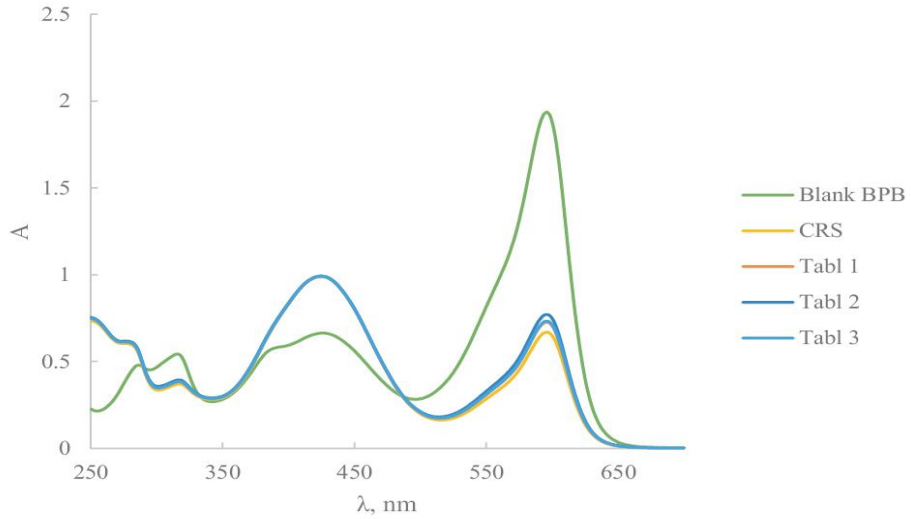


Figure 2. Absorption spectra of the valsartan–BPB complex.

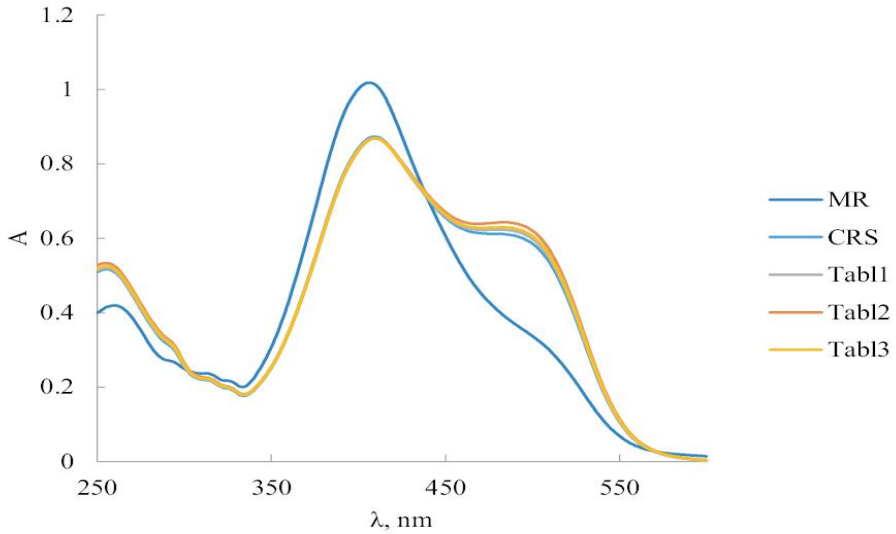


Figure 3. Absorption spectra of the valsartan–MR complex.

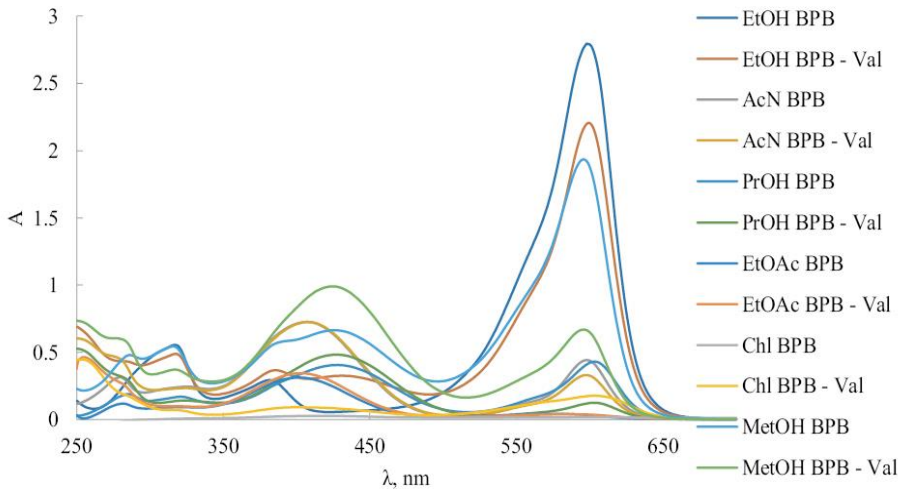


Figure 4. Absorption spectra of BPB and the valsartan–BPB complex in various solvents.

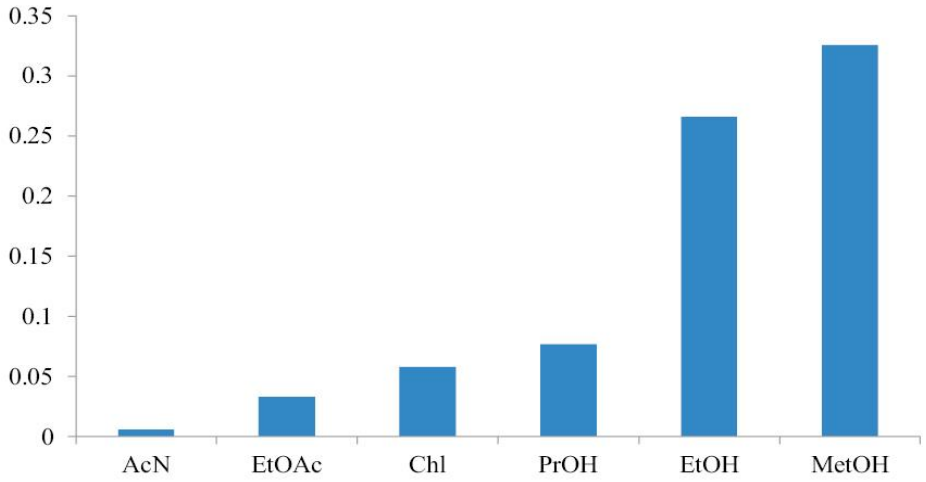


Figure 5. Absolute ΔA values representing the difference between the absorption of the valsartan–BPB complex and that of BPB alone in various solvents.

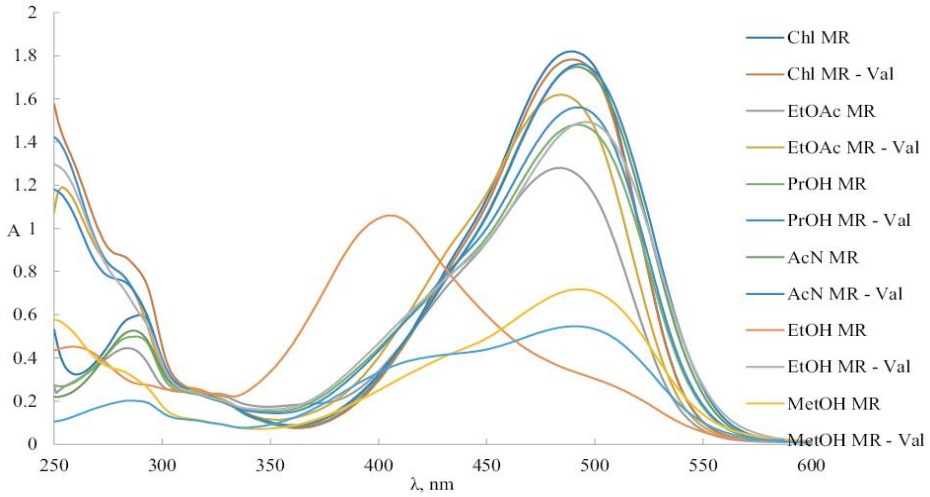


Figure 6. Absorption spectra of MR and the valsartan–MR complex in various solvents.

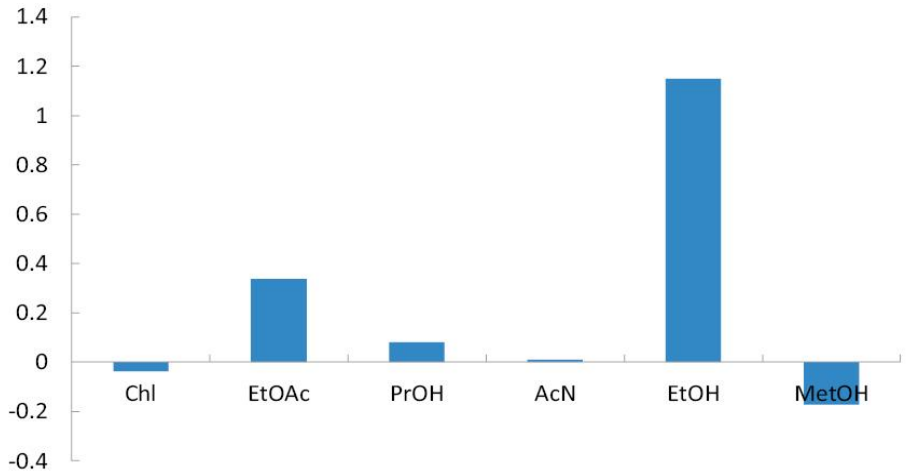
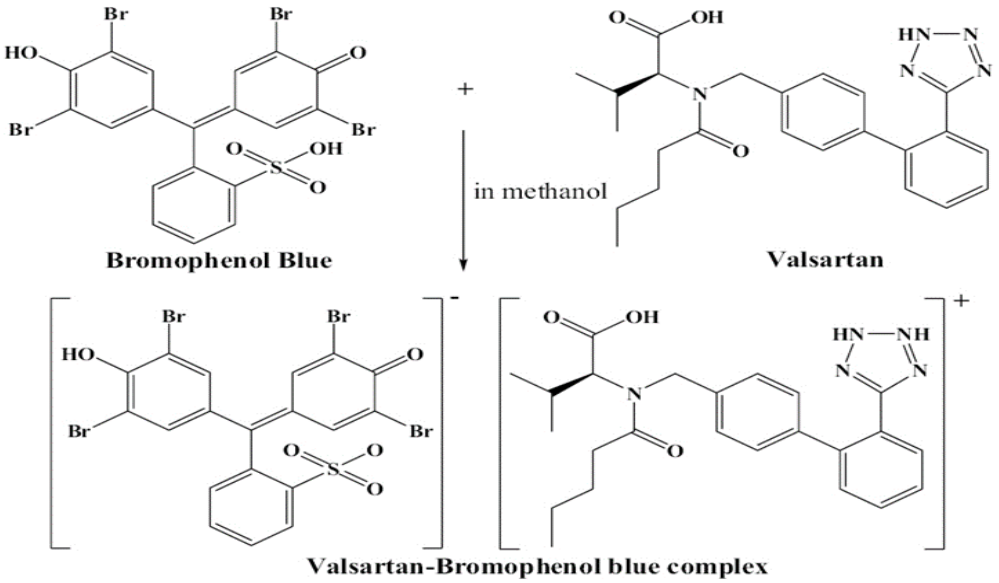
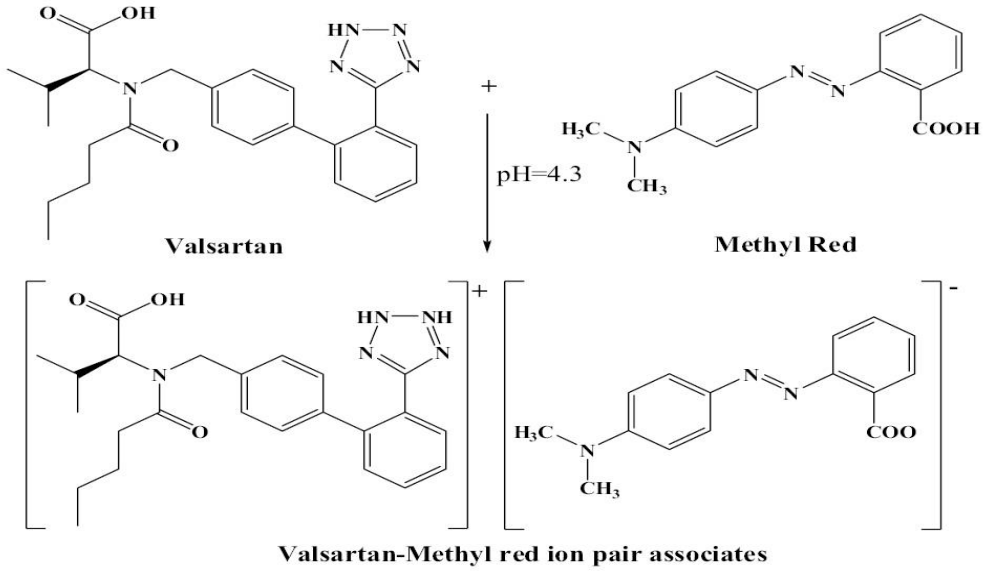


Figure 7. Absolute ΔA values showing the difference between the absorption of the valsartan–MR complex and that of MR alone in various solvents.



Scheme 1. Proposed reaction pathway illustrating the formation of the valsartan–bromophenol blue (BPB) complex.



Scheme 2. Proposed reaction pathway illustrating the formation of the valsartan–methyl red (MR) ion-pair complex.

To achieve rapid and quantitative generation of stable, intensely colored ion-pair complexes, optimization of the experimental conditions was essential. The highest absorbance values were recorded in methanol for the valsartan–BPB system and in ethanol for the valsartan–MR system, while solvents such as chloroform, propanol, acetonitrile, and ethyl acetate were unsuitable for complex formation. The absorption spectra of these systems in various solvents are shown in **Figures 4 and 6**, and the corresponding absolute ΔA values, representing the difference in absorbance between the dye–drug complexes and the individual dyes, are depicted in **Figures 5 and 7**.

To evaluate the analytical sensitivity of valsartan's interactions with BPB and MR, parameters such as molar absorptivity, specific absorbance, and Sandell's sensitivity were calculated. The molar absorption coefficient (ε) was determined to be 4.43×10^3 for BPB and 2.36×10^4 for MR; the specific absorbance (a) was 1.07×10^{-1} for BPB and 5.4×10^{-1} for MR; and the Sandell coefficient (Ws) was 9.83×10^{-2} for BPB and 1.84×10^{-2} for MR. These findings demonstrated that the valsartan–MR reaction exhibits higher analytical sensitivity than the valsartan–BPB reaction, as evidenced by a greater molar absorptivity and a lower detection threshold.

The stoichiometry of both reactions was established using Job's method of continuous variation (Job, 1936). Equimolar solutions of valsartan (1.3×10^{-3} M with BPB and 3.7×10^{-3} M with MR) were prepared, and results indicated a 1:1 stoichiometric ratio between the dye and drug for both complexes. These findings align with the proposed reaction mechanisms illustrated in Schemes 1 and 2 **(Figures 8 and 9)**.

Method validation

Linearity

Key analytical parameters, including Beer's law limits, molar absorptivity, detection limit, regression equations, and correlation coefficients, were derived through least-squares analysis in accordance with [12] guidelines. A linear correlation between absorbance at λmax and valsartan concentration was observed within the ranges of 8–24 µg/mL for BPB and 4–20 µg/mL for MR. The corresponding regression equations were $y = 0.0102x + 0.1636$ at 424 nm (BPB) and $y = 0.0222x – 0.0063$ at 494 nm (MR). High correlation coefficients ($R^2 = 0.9988$ for BPB and $R^2 = 0.9991$ for MR) and minimal intercept values confirmed the excellent linearity and adherence to Beer's law. The calibration plots are shown in **Figures 10 and 11**.

Limits of detection and quantification

In accordance with ICH recommendations, the limits of detection (LOD) and quantification (LOQ) were calculated using the standard deviation of the response and the slope of the calibration curve, applying factors of 3.3 and 10, respectively. The calculated LOD and LOQ were 1.03 µg/mL and 3.43 µg/mL for BPB, and 0.68 µg/mL and 2.26 µg/mL for MR, respectively.

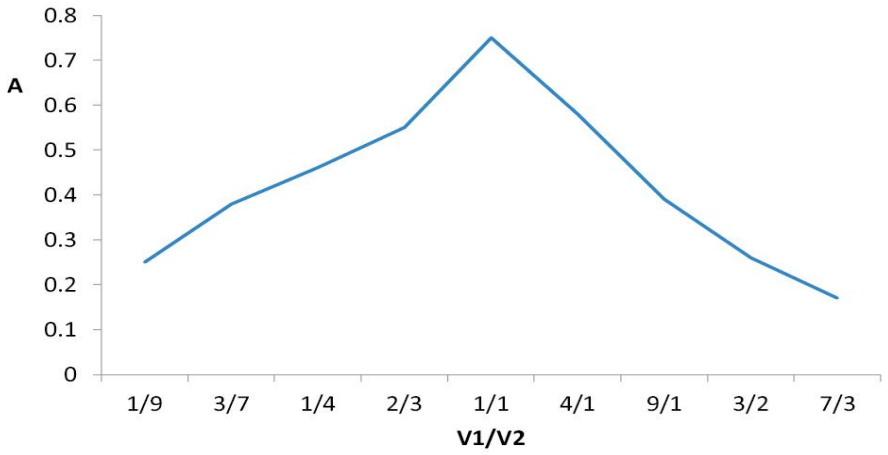


Figure 8. Job's plot illustrating the molar ratio of the valsartan–BPB ion-pair complex formation.

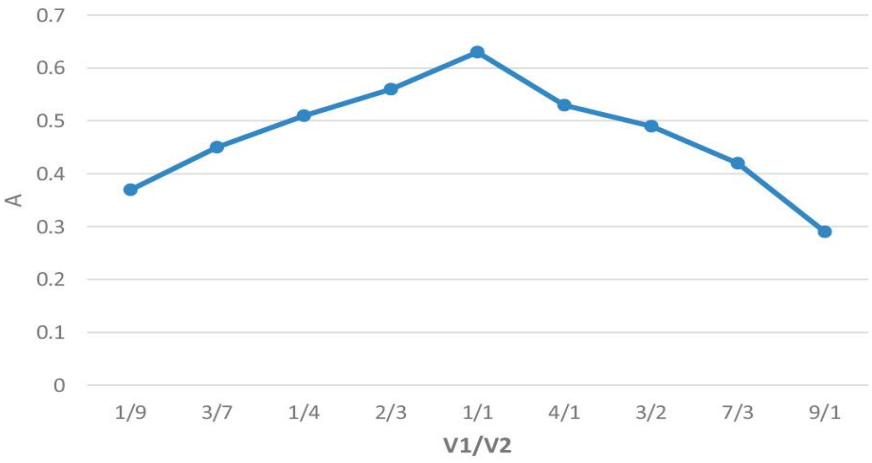


Figure 9. Job's plot illustrating the molar ratio of the valsartan–MR ion-pair complex formation.

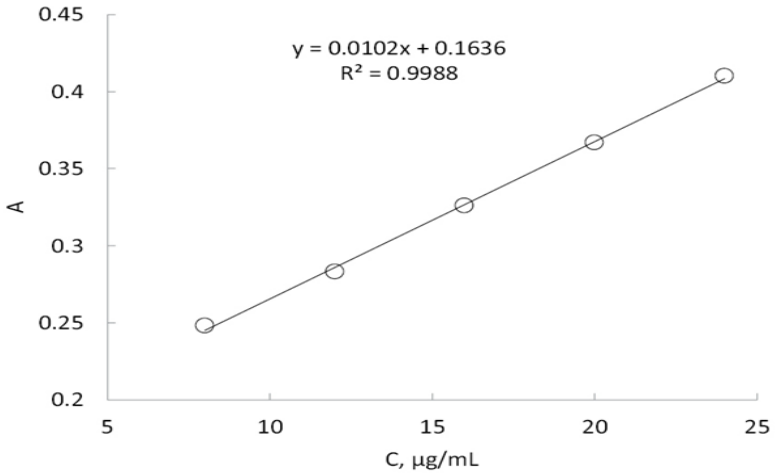


Figure 10. Calibration plot for valsartan determination using BPB.

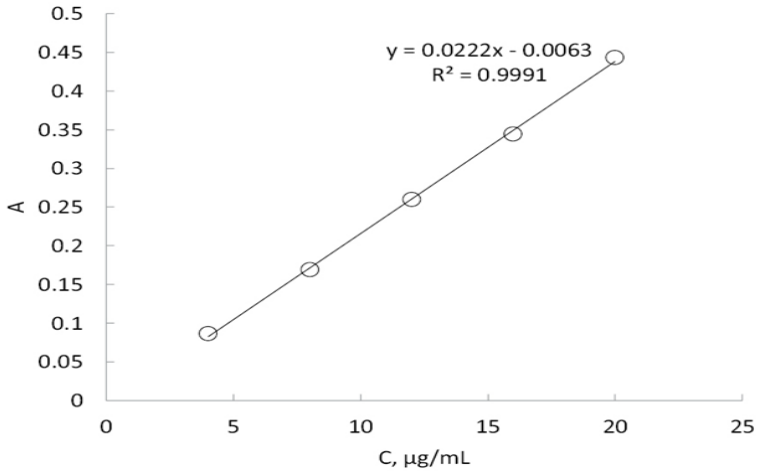


Figure 11. Calibration plot for valsartan determination using MR.

Selectivity

The selectivity of the developed spectrophotometric methods was evaluated using an artificial mixture to verify that the recorded absorbance originated solely from valsartan. A synthetic formulation containing valsartan (160 mg) along with common excipients—lactose monohydrate, microcrystalline cellulose, povidone, croscarmellose sodium, colloidal anhydrous silica, magnesium stearate, hypromellose, and titanium dioxide—was prepared and processed following the same extraction and analytical procedures used for tablets. The results of five replicate analyses (n = 5) demonstrated recovery rates of 100.14 ± 1.03% for a 16 µg/mL valsartan concentration (BPB method) and 100.27 ± 1.15% for a 12 µg/mL concentration (MR method). These outcomes confirmed that excipients had no interfering effect on valsartan quantification.

Precision and accuracy

To assess precision, intra-day and inter-day analyses were conducted by measuring calibration standards at three different concentration levels, five times each, within a single day and across five consecutive days. The resulting relative standard deviation (RSD%) values demonstrated excellent repeatability and reproducibility, confirming good precision **(Table 1)**. Accuracy—expressed as the percentage relative error between the experimental and theoretical valsartan concentrations—also indicated strong agreement, as shown in **Table 1**, supporting the reliability of the developed analytical methods.

Robustness

Robustness testing was performed during the optimization phase to determine the stability of the spectrophotometric methods and to identify factors influencing optical density, such as reagent volume and solution stability. Variations in BPB and MR volumes within ±10% showed no significant impact on analytical

results **(Figures 12 and 13) (Tables 2 and 3)**. Furthermore, the prepared solutions maintained stability for at least 45 minutes, as demonstrated in **Figures 14 and 15**.

Table 1. Intra-day and inter-day accuracy and precision.

Method	Valsartan taken, µg/mL	Intra-day accuracy and precision			Inter-day accuracy and precision		
		Valsartan found, µg/mL	RE, %	RSD, %	Valsartan found, µg/mL	RE, %	RSD, %
BPB	8	8.03	0.59	1.03	7.91	0.53	1.08
	15	15.05	0.64	1.13	15.09	0.67	1.17
	24	23.87	1.14	1.17	24.13	0.86	1.23
MR	4	4.05	0.63	1.12	4.05	0.56	1.05
	12	11.94	0.56	1.06	12.11	0.64	1.09
	20	20.11	0.69	1.09	19.92	0.65	1.11

RE: Relative error; RSD: Relative standard deviation

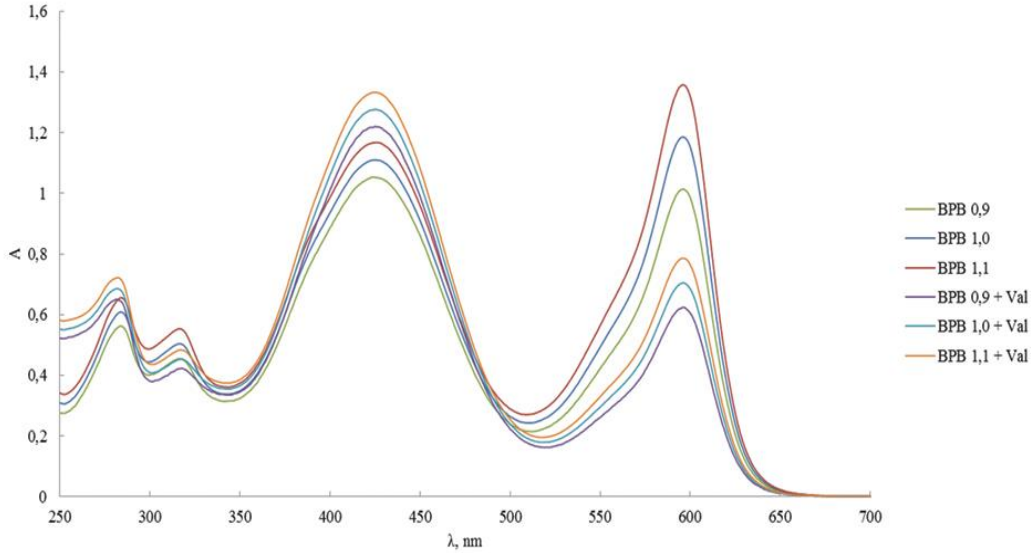


Figure 12. Absorption spectra of BPB and the valsartan–BPB complex under varying amounts of added BPB.

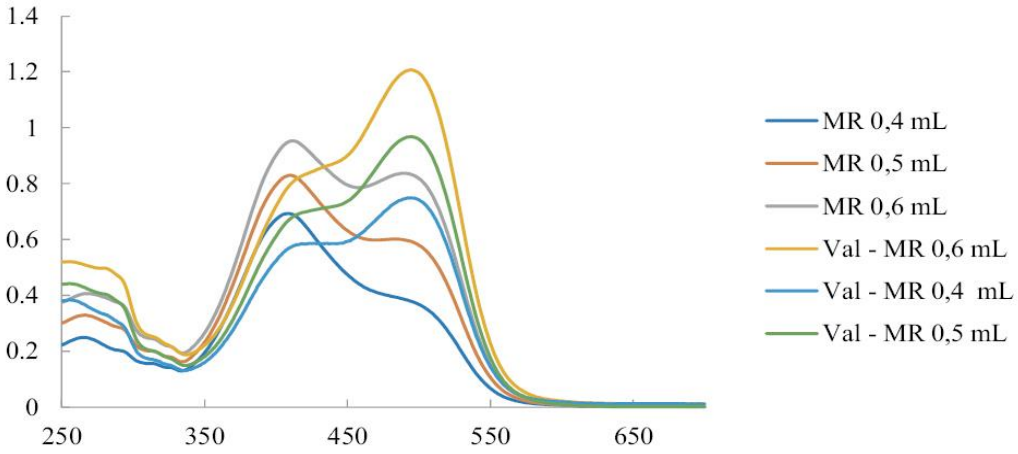


Figure 13. Absorption spectra of MR and the valsartan–MR complex under varying amounts of added MR.

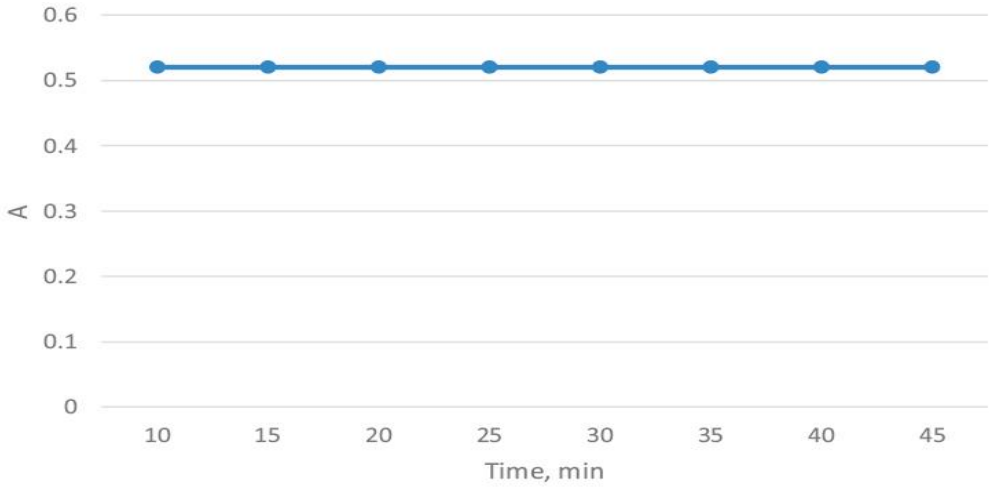


Figure 14. Time-dependent absorption profile of the valsartan–BPB reaction product.

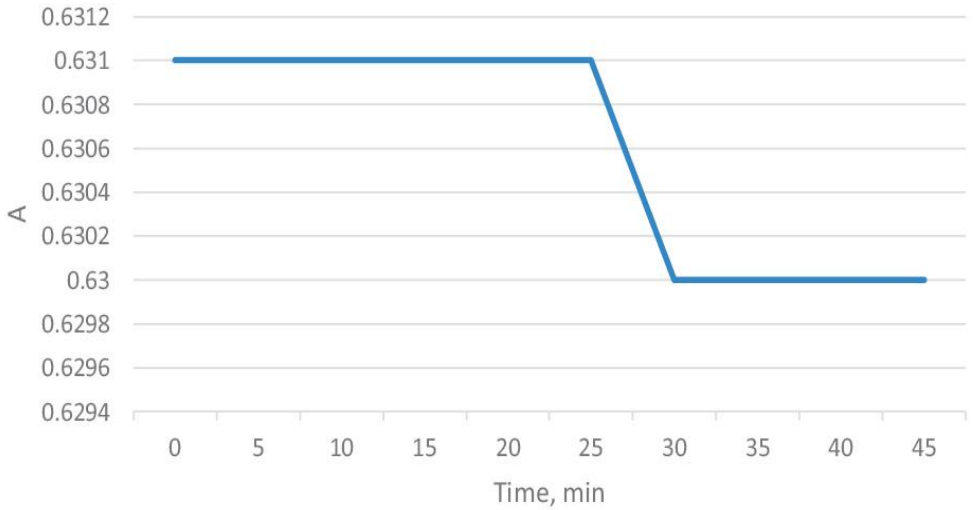


Figure 15. Time-dependent absorption profile of the valsartan–MR reaction product.

Table 2. The results of the change in optical density from the amount of added BPB.

Amount of added BPB, mL	**Δ A**
0.9	0.164
1.0	0.165
1.1	0.166

Table 3. The results of the change in optical density from the amount of added MR.

Amount of added MR, mL	**Δ A**
0.4	0.370
0.5	0.374
0.6	0.373

Table 4. Determination of valsartan formulation by the proposed methods.

Tablet brand name	**Label claim, mg/tablet**	**Found (label claim ± SD)**, %	
		BPB	**MR**
Valsartan KPKA	160	100.045 ± 0.538	100.035 ± 0.324

		t=2.11	t=2.48
		F=4.27	F=3.04
Valsartan Sandoz	160	100.028 ± 0.345	100.039 ± 0.482
		t=2.29	t=2.12
		F=3.35	F=3.51
Valsartan-Teva	160	100.045 ± 0.485	101.004 ± 0.567
		t=2.31	t=2.04
		F=3.86	F=3.29

The tabulated t-value at a 95% confidence level is 2.57, and the tabulated F-value at a 95% confidence level is 6.39.

Table 5. Analytical eco-scale for greenness assessment of the developed spectrophotometric methods.

Parameters	**Penalty points (PP)**	
	BPB	**MR**
Reagents Methanol	4	–
Ethanol	–	4
Bromphenol blue	1	–
Methyl red	–	1
Energy consumption	1	1
Occupational hazards	0	0
Waste	5	5
Total penalty points (PP)	11	11
Analytical Eco-scale score	89	89
Comment	Excellent green analysis	Excellent green analysis

Application to pharmaceutical formulation

The developed spectrophotometric methods were successfully applied to quantify valsartan in tablets from three different commercial products. As shown in **Table 4**, the results indicate no significant differences between the methods. The calculated Student's t- and F-values at a 95% confidence level were lower than the corresponding theoretical values, confirming a strong agreement between the outcomes obtained using the proposed approaches.

Analytical Eco-Scale for greenness assessment

Modern pharmaceutical analysis emphasizes adherence to green chemistry principles, which should be considered when developing analytical techniques for the determination of valsartan in both pure substances and pharmaceutical formulations [13-21]. The results summarized in **Table 5** demonstrate that both developed methods achieved an excellent eco-scale score of 89, reflecting their high environmental compatibility.

Conclusion

Two rapid, simple, and environmentally friendly non-extractive spectrophotometric methods were established for the determination of valsartan in tablet dosage forms, based on ion-pair complex formation with bromophenol blue (BPB) and methyl red (MR). Optimal spectrophotometric conditions were defined, and the methods require only a single reagent and inexpensive instrumentation. The limits of quantification for these methods (3.43 µg/mL for BPB and 2.26 µg/mL for MR) are considerably lower than those of existing techniques. These advantages support the routine application of the proposed methods in quality control laboratories.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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