Research Article

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Smart mathematically filtered UV spectroscopic methods for quality assurance of rosuvastatin and valsartan from formulation

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Abstract: Valsartan and rosuvastatin together in a binary form have been utilized to reduce hypertension and hyperlipidemia to control cardiovascular complications. This study depicts the simple three mathematically manipulated UV spectroscopic techniques for the estimation of rosuvastatin and valsartan in the formulation. The first method is simple UV absorption at 310 nm by RST and the first derivatization method for VTN. Determining the magnitude difference of a ratio spectrum at two identified wavelengths is the second approach, and determination of the magnitude of the first derivatives of the ratio spectra of RST and VTN constitute the third technique. The selection of wavelengths, divisor concentrations, and peak amplitudes were optimized and validated. The straight line was constructed in the range of 1–30 and 2–25 µg/ml for RST and VST by the normal and first derivatization method. By using the magnitude difference and magnitude of first derivative ratio spectra approaches, the concentrations of 1–12 and 2–25 µg/ml for RST and VTN, respectively, displayed a straight line. The limit of quantification was less than 1 µg/ml for RST and less than 2 µg/ml for VTN. It was eventually found that the accuracy, expressed as a percentage recovery, ranged between 98.94 and 99.55% for RST and 100.36 and 101.08% for VTN. The % RSD did not exceed 1.82 and 1.91 for RST and VTN, respectively. The three techniques were used to accurately measure RST and VTN in their binary formulations and physically mixed solutions, and the results were statistically compared to the previously published HPLC technique. The outstanding recovery achieved by using the authentic standard addition approach validated the methods' supplemental accurateness. The Analytical Greenness and Red Green Blue procedures verified the eco-friendliness of the suggested UV spectroscopic approaches, which were also found to be superior to the documented HPLC methods.

Keywords: rosuvastatin, valsartan, UV derivative technique, spectroscopy, formulation validation

1 Introduction

Worldwide more than a billion people are suffering from high blood pressure; further, this number is increasing, making the major chronic disease responsible for high morbidity and mortality. Heart attack, myocardial infarction, and stroke are merely some of the cardiovascular problems that are caused by hypertension [1–3]. Valsartan chemically (2*S*)-3-methyl-2-[pentanoyl-[[4-[2-(2*H*-tetrazol-5-yl)phenyl] phenyl]methyl]amino] butanoic acid (VTN, Figure 1a), an angiotensin II (ANG II) receptor type 1 inhibitor, prescribed for the management of hypertension and other cardiovascular complications. Valsartan lowers blood pressure by resisting the vasoconstriction and aldosterone-secreting effects of ANG II, by specifically preventing ANG II from binding to the angiotensin 1 receptor in vascular smooth muscle [4].

Dyslipidemia is an increased amount of low-density cholesterol and triglycerides and a decline in high-density cholesterol, which causes deposing and hardening of arteries. Hence, controlling the blood pressure and maintaining the normal lipid profile are essential to reduce cardiovascular disorders [5].

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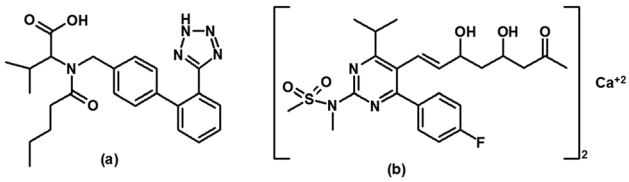

Figure 1: Structural formula of valsartan (a) and rosuvastatin calcium (b).

Rosuvastatin, chemically 3*R*,5*S*,6*E*)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl)amino]pyrimidin-5-yl])-3,5-dihydroxyhept-6-enoicacid hemicalcium salt (RST, Figure 1b), is an orally active lipid-lowering statin used for the treatment of cardiovascular diseases produced due to hypercholesterolemia and mixed dyslipidemia. In the liver, it inhibits the formation of cholesterol by inhibiting the creation of mevalonic acid from 3-hydroxy-3-methyl-glutaryl coenzyme A, a limiting step of the cholesterol synthesis. This enhances the catabolism of low-density protein (LDP) by increasing the LDP receptor on the hepatic cell membranes, thus reducing the concentration of LDP and very low-density lipoprotein in the blood [6–8].

A binary mixture of valsartan and rosuvastatin proved effective in reducing hypertension and hyperlipidemia to control cardiovascular complications [9].

Owing to the medication of choice's extreme importance, numerous analytical techniques have been developed for the identification of VTN and RST, both on their own and in combination with other medications. Upon examining the available literature, it was observed that the majority of methods mentioned for the quantification of VTN are spectrophotometric [10,11], spectrofluorometric [12], and HPLC procedures [13–18]. Other methods reported are the determination of the most common impurities present in the sartans along with the VTN using liquid or gas chromatography with a mass detector. The reported methods for the analysis of RST and its impurities include HPLC and UPLC [19–22]. The estimation of RST along with other cardiovascular agents comprises spectrophotometric [23–26], HPTLC [27], HPLC [28–30], and UPLC [31,32] procedures in medicines and physiological samples.

Dyade and Sawant established HPLC methods for the concurrent estimation of VTN and RST from the formulation [33] and plasma [34]. However, there is currently no published spectroscopic technique for the determination of VTN and RST simultaneously from formulations. Spectrophotometric methods of analysis are simple, accurate, and most extensively used analytical methods, due to their eco-friendly and economical nature compared to liquid chromatographic technique. A maximum number of pharmaceutical compounds show incredible UV absorption owing to aromatic or hetero-aromatic structures, making easy analysis of mono medicine formulations. However, direct analysis of multicomponent formulations showing overlapped spectra is difficult without chemical separation. Further, different spectral manipulation techniques using soft wares are established for the concurrent quantification of medicines with several drugs by separating only the spectra [35–39].

The mathematically manipulated spectrophotometric assay technique, which is widely available in most analytical laboratories, offers high simplicity, quick analysis, and minimal use of organic solvents, eliminates sample pretreatment processes, and is inexpensive. Analytical Greenness (AGREE) [38–40] and White Analytical Chemistry, Red Green Blue (RGB) [38,41,42] tools were used to assess the greenness and whiteness of the spectroscopic techniques, respectively. This focuses our attention on creating an affordable, safe for the environment, straightforward, sensitive UV spectroscopic technique for concurrently determining the compounds under investigation. The proposed environmentally friendly UV spectroscopic techniques have been validated and used to ensure the quality of the mixture made in the lab and the VTN and RST binary formulation.

2 Experimental methods

2.1 Materials and reagents

Authentic standards rosuvastatin calcium (99.5%) and valsartan (99.2%) have been secured from Biokemix India Limited (Hyderabad, India). RST 20 mg (Rosuvas 20) and 40 mg (Rosuvas 40) tables a product of Sun Pharmaceutical Industries Limited and valsartan 80 mg (Diovan 80 mg) and 160 mg (Diovan 160) tablets a products of Novartis India Limited were purchased at the native medical store. Ethanol (99.9%) analytical grade has been acquired from Scharlau (Sentimenat Spain).

2.2 Instrument and software

UV–Vis spectrophotometer (Shimadzu 1700, Tokyo, Japan) was utilized for recording the absorption spectra of analytes. The spectrophotometer was connected to a PC installed with UVProbe 2.21 software. Two similar 1 cm quartz cuvettes have been utilized to scan blank and compound solutions. A spectrophotometer has been set for rapid scanning with a sampling interval of 0.1 nm and an opening breadth of 2 nm.

2.3 Preparation of authentic standard solutions

A perfectly measured 50 mg of RST and VTN has been dissolved using 25 ml of ethanol in a 50-ml volumetric flask. With ethanol, the remaining proportion was brought up to the 50 ml threshold. The operational authentic solutions were arranged using previous solutions by adding distilled water. The refrigerator was used to keep the solutions and thawed to room temperature before use.

2.4 Procedure for the calibration curve

2.4.1 Zero and first derivative spectroscopic method (^{0}D and ^{1}D methods)

The calibration curve for RST was prepared using 1–30 µg/ml solutions by transferring an accurate quantity of RST standard solution. The solutions were scanned in the range of 200–400 nm, and absorption was determined at 310 nm and plotted against the corresponding quantity of RST. The calibration curve for VTN was prepared using 2–25 µg/ml solutions by transferring the required quantity of VTN authentic solution. The solutions were exposed to absorption and subjected to derivatization of first order employing 4 nm as $\Delta\lambda$ and an increment multiplier of 10. Apex magnitude was recorded at 223.8 nm and a straight line has been drawn against the corresponding concentration of VTN.

2.4.2 Ratio difference absorption (RDA) method

Authentic standards of RST and VTN in the concentration of 1–12 and 2–25 µg/ml were prepared separately by shifting the sufficient quantity of respective analyte solutions. Ultraviolet absorption spectra were obtained in the UV region. To obtain the ratio spectrum of RST, the absorption spectrum of 12 µg/ml VTN solution was utilized to divide the RST absorption spectra. The magnitude of the spectra at 343.6 nm was subtracted from 309.8 nm to find the peak magnitude difference and plotted against the corresponding concentration of RST. To get the ratio spectrum of VTN, the absorption spectrum of the 6 µg/ml solution of RST was utilized to divide the absorption spectra of VTN in a similar manner. The peak measurement at 245.2 nm was subtracted from the magnitude at 214.0 nm to find the peak magnitude variance and drawn against the corresponding concentration of VTN.

2.4.3 First derivative ratio spectra method (FDR)

Using 2 nm as $\Delta\lambda$ and an increment coefficient of 10, the aforementioned documented ratio spectra have been derivatized into the first-order spectra. Apex magnitude has been computed at 302.6 and 231.5 nm from respective RST and VTN FDR spectra and plotted against corresponding concentrations of RST and VTN, respectively. Additionally, regression equation and coefficient of determination were generated.

2.5 Application to manually blended solutions

Manually blended solutions have been arranged by adding a sufficient quantity of standard solutions of RST and VTN to a 5 ml volumetric flask to get a final concentration of 6:25, 6:24, 5:24, 3:12, and 10:20 µg/ml. After going through the scanning across 200 and 400 nm, the outcomes have been retained in the computer. Employing the aforementioned approach, first-order, ratio, and first-derivative ratio spectra was produced, and the appropriate equations of the straight line were used to calculate the quantities of RST and VTN.

2.6 Application to pharmaceutical sample

A combined formulation containing RST and VTN was not available in the local market; hence, a formulation containing RST 20 + VTN 80 mg and RST 40 + VTN 160 mg has been arranged by combining the tablet powder of RST and VTN. The median mass of the 20 RST and VTN tablets was determined by weighing each one carefully. RST 20 mg and VTN 80 mg tablets were powdered together and mixed properly and 10 ml of ethanol had been utilized to dissolve the tablet mixture, equal to 2 mg of RST and 8 mg of VTN. Similarly, a sample solution was prepared from RST 40 mg and VTN 160 mg tablets. To achieve the concentration of the aforementioned solutions within the straight line, they were further attenuated by water, and the absorbance spectra were taken between 200 and 400 nm in wavelength. Using the above-described approach, first-order, ratio, and first-order ratio spectra were produced, and the appropriate straight-line formulas were used to calculate the quantities of RST and VTN.

3 Results and discussion

Both the analytes RST and VTN are highly aromatic structure and hence showed a complete overlap of UV

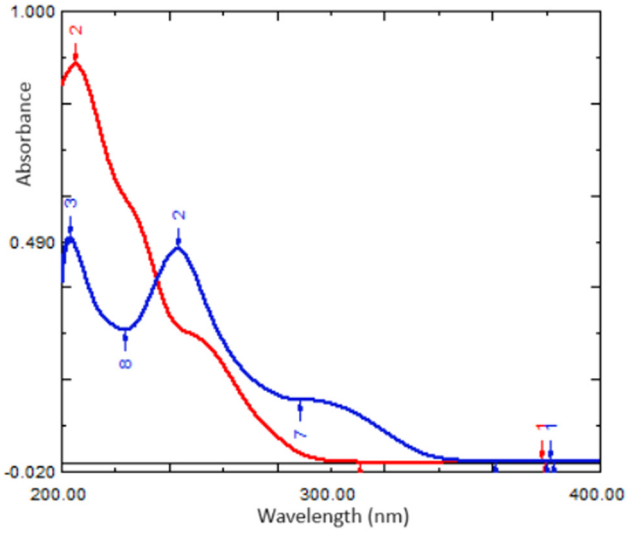

Figure 2: UV absorption spectra of VTN (red) and RST (blue).

absorption spectra between 200 and 300 nm (Figure 2). Unfortunately, it was challenging and difficult to quantify both analytes using the simple UV absorption approach. Hence, different UV absorption manipulation techniques such as derivatization, and generation of ratio spectra was adopted for the simultaneous quantification of analytes under investigation.

3.1 Zero and first derivative absorption method

It is evident from Figure 2 that RST showed good absorption after 300 nm, whereas VTN had no absorption; hence, RST was quantified by gauging the apex magnitude at 310 nm (Figure 3a). However, VTN showed complete overlap with the RST absorption spectra; hence, the first-order derivatization technique of zero intersection point [35–37] was implemented for the quantification of VTN. The apex magnitude of first-order spectra of the mixture measured at zero intersection of one of the compounds represents the absorption of another compound. The first derivative spectra of VTN showed three wavelength points 290.4, 242.8, and 223.8 nm at which RST had zero absorption (Figure 3b). However, the peak magnitude of VTN at wavelength 223.8 nm showed a good linearity range and the peak amplitude and reproducibility were better. 2, 4, and 8 nm have been envisaged as $\Delta\lambda$ during the transformation of VTN zero-order spectra into first derivative spectra, with 2 nm, the first-order spectra showed noise, whereas with 8 nm the specificity was low. In addition, scaling factor 10 provided good peak intensity even at low concentrations. Further, a series of VTN solution spectra were recorded between 200 and 400 nm and altered into first derivative spectra by means of 4 nm as $\Delta\lambda$ along with multiplication coefficient 10. Apex magnitude at 223.8 nm was measured and plotted against the concentration of VTN. The individual and combination first derivative spectra with identical quantities of VTN revealed the same peak magnitude at 223.8 nm.

3.2 Ratio spectra absorption difference method

Apex magnitude discrepancy measurements at two preferred wavelengths of the ratio spectrum are a well-

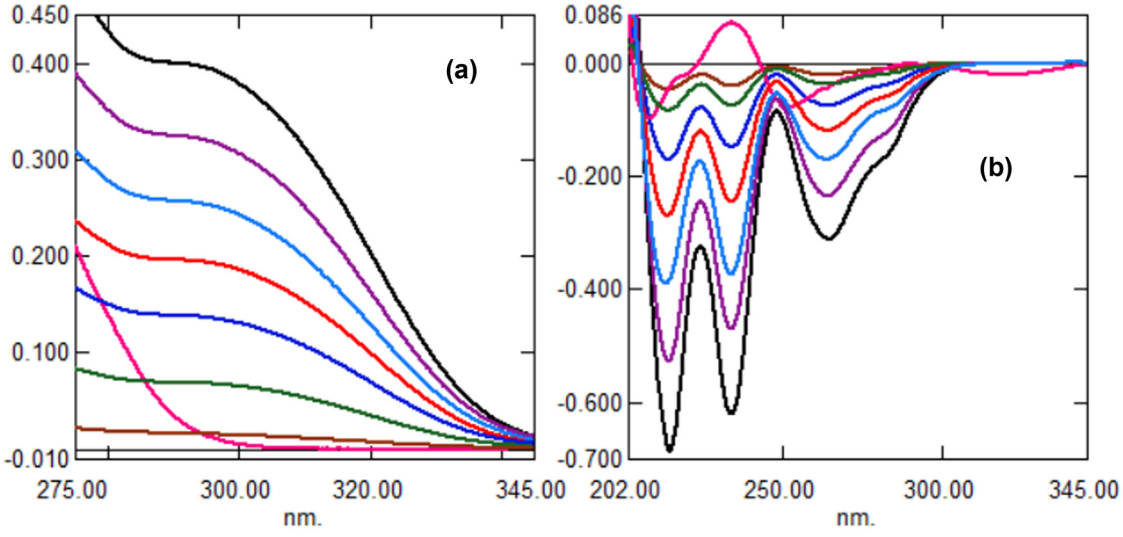

Figure 3: Normal zero order spectra of RST (1–30 µg/ml) (a) and first-order derivative spectra of VTN (2–25 µg/ml) (b).

established technique for the quantification of multicomponent formulation showing completely overlapped UV absorption spectra [38,39]. Ratio spectra is generated by division of the combined spectra by one compound spectrum, which eliminates the divisor component allowing quantifying another component of the mixture. The selection of divisor concentration has to be envisaged to get reproducible and accurate results. Different concentrations of RST and VGT were tried as divisor spectra, with low concentration the peak amplitude of ratio spectra was very high with high noise, whereas with high concentration peak height was less with less noise spectrum. However, the 6 µg/ml solution spectrum of RST and the 12 µg/ml solution spectrum of VTN produced good, reproducible ratio spectra. A 4 nm had been preferred as $\Delta\lambda$ to smoothen the ratio spectra. An array of RST spectra ranging from 1 to 12 µg/ml has been divided by the 12 µg/ml VTN solution spectrum, to generate ratio spectra of RST (Figure 4a). In the same manner, to create ratio spectra of VTN, a range of VTN spectra between 2 and 25 µg/ml had to be divided by the RST solution spectrum containing 6 µg/ml (Figure 4b).

A mixture's ratio spectrum usually shows up at a higher level than the baseline when compared to the ratio spectra of individual analytes. However, the magnitude variance at selected two wavelengths of ratio spectra of the mixture and pure remains the same. The two wavelengths selected were at 343.6 and 309.8 nm for RST ratio spectra, whereas 214.0 and 245.2 nm for VTN ratio spectra. The amplitude difference was determined and outlined in relation to the relevant concentration of RST (Figure 4c) and VTN (Figure 4d) to generate calibration curves. Further,

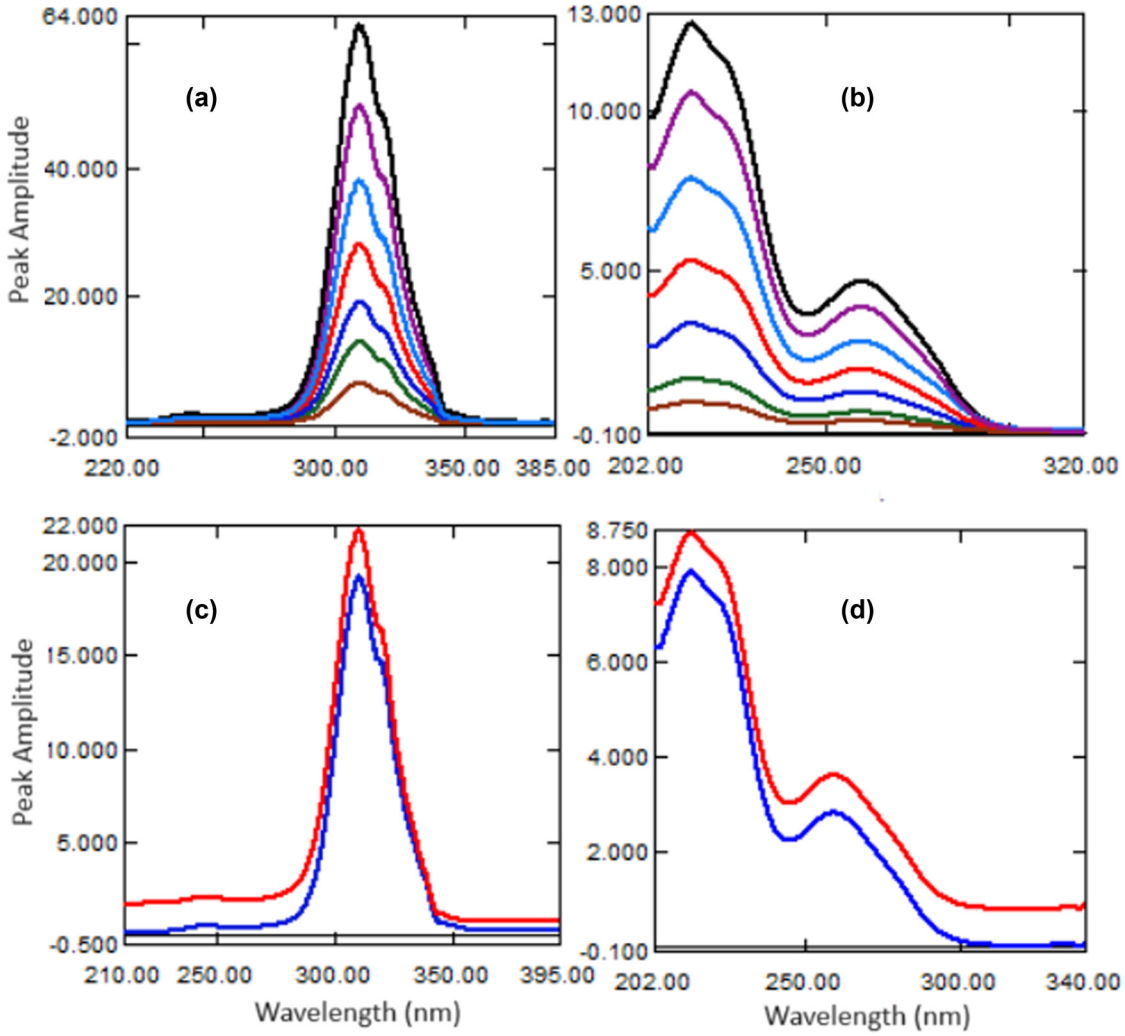

Figure 4: Ratio spectra of RST (1–12 µg/ml) using 12 µg/ml VTN spectrum as divisor (a). Ratio spectra of VTN (2–25 µg/ml) using 6 µg/ml RST spectrum as divisor (b). Comparison of ratio spectra of mixture (red) and pure (blue) RST (c) and VTN (d).

the individual and combination ratio spectra with identical quantities of analytes revealed the same magnitude difference.

3.3 First derivative of the ratio spectra method

Derivatization of normal spectra enhances the resolution of overlapped spectra and eliminates unnecessary background absorption along with an increase in sensitivity and selectivity [37–39]. In this study, the ratio spectra of combination and pure can be converted to first derivative spectra to eradicate the effect of one of the analytes. Further derivatization provides many satellite peaks and refractory dips which can be utilized for quantification of analytes. The first derivative spectra of RST (Figure 5a) displayed three satellite peaks at 237.4, 297.6, and 302.6 nm and five refractory dips at 314.2, 323.4, 327.7, 333.9, and 339.9 nm. The ratio first-order derivatization spectrum of VTN (Figure 5b) revealed two satellite peaks at 207.2 and 251.7 nm and five refractory dips at 217.4, 231.3, 270.3, and 283.3 nm. However, satellite peaks 302.6 and 231.5 nm of RST and VTN, respectively, demonstrated high reproducibility and accuracy hence selected for further analysis. The individual and combination first derivative spectra with identical quantities of RST (Figure 5c) and VTN (Figure 5d) revealed the same peak magnitude.

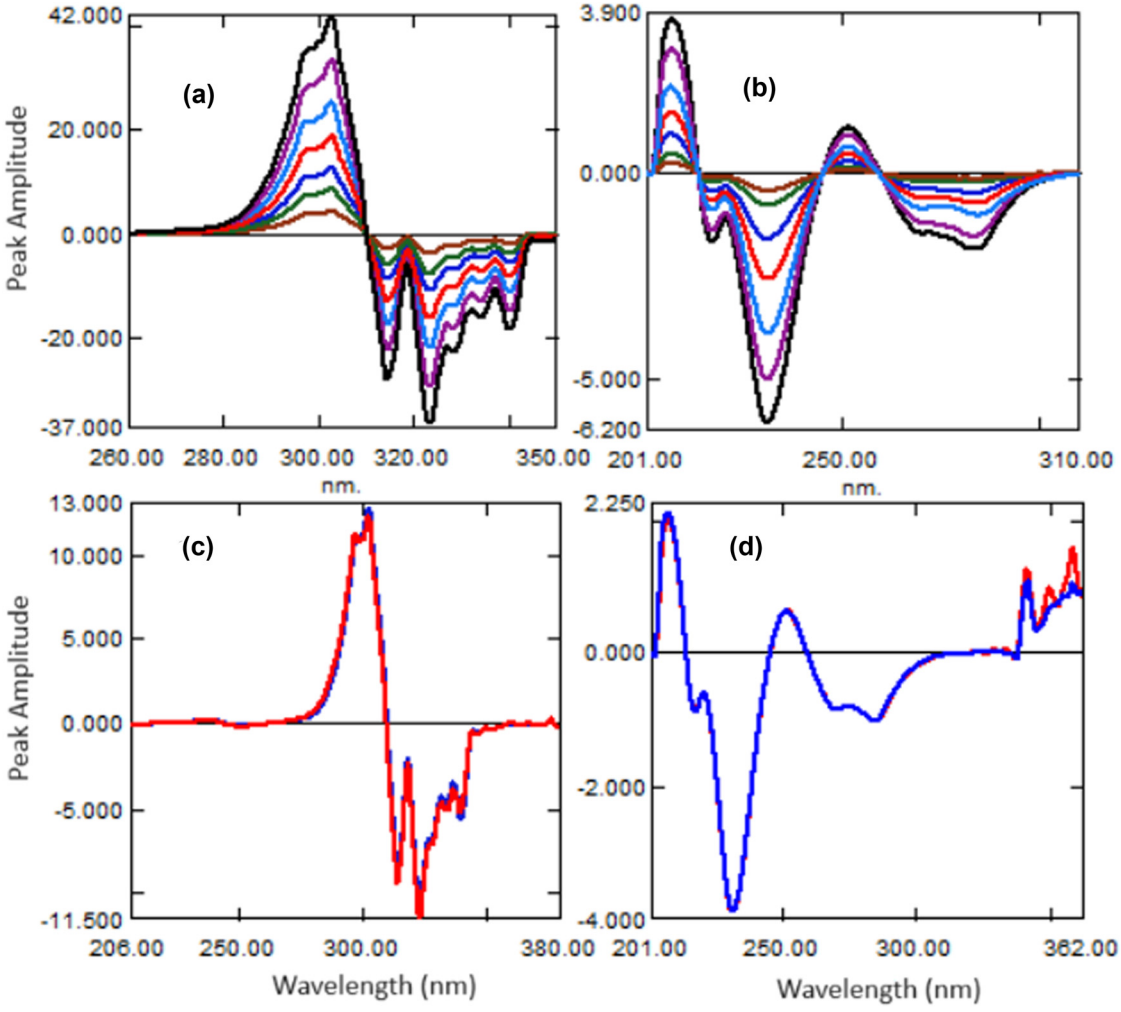

Figure 5: First derivative of ratio spectra of RST (1–12 µg/ml) using 12 µg/ml VTN spectrum as divisor (a). First derivative of ratio spectra of VTN (2–25 µg/ml) using 6 µg/ml RST spectrum as divisor (b). Comparison of the first derivative of ratio spectra of mixture (red) and pure (blue) RST (c) and VTN (d).

Table 1: Outcomes of validation features for RST and VTN

Factors	^{0}D and ^{1}D spectra method		RDA		FDR	
Analytes	**RST**	**VTN**	**RST**	**VTN**	**RST**	**VTN**
Wavelength (nm)	310.0	223.8	309.8–343.6	214.0–245.2	302.6	231.5
Linearity (μg/ml)	1–30	2–25	1–12	2–25	1–12	2–25
LOD (μg/ml)	0.22	0.47	0.24	0.39	0.31	0.59
LOQ (μg/ml)	0.65	1.46	0.71	1.15	0.94	1.78
Slop	0.010	0.011	4.797	0.370	3.147	0.246
Intercept	0.002	0.004	1.519	0.225	1.766	0.172
Correlation coefficient (r^2)	0.9996	0.9996	0.9997	0.9999	0.9989	0.9998
Accuracy (mean % ± RSE)	98.94 ± 1.17	100.62 ± 1.42	99.55 ± 0.86	101.08 ± 0.92	99.39 ± 1.43	100.36 ± 0.75
Precision (%RSD)						
Repeatability	1.82	1.37	1.07	0.97	0.83	1.46
Within day	0.95	0.73	1.22	1.13	1.43	1.28
Between days	1.09	0.86	1.47	1.51	1.04	1.91

RSE: relative standard error; RSD: relative standard deviation.

3.4 Validation of UV spectroscopic methods

3.4.1 Straight line and sensitivity

Using the established UV spectroscopic approaches, both analytes have been evaluated for the setting up of a straight line and the linearity range for all three methods was 1–30 and 1–12 μg/ml for RST and 2–25 μg/ml for VTN. The straight-line fitting formula correlation coefficients and other validation factors are tabulated (Table 1). By calculating the limit of detection and quantification implementing the International Conference of Harmonization recommendations, the sensitivity of the offered approaches has been investigated. The LOD had been computed *via* 3.3 times of ratio of slope to the standard deviation of intercept, whereas LOQ was 10 times of ratio of slope to the standard deviation of intercept. The linearity range and LOQ remained sufficient enough for the determination of the analytes from the formulations (Table 1).

3.4.2 Precision and accuracy

The precision indicates the repeatability and reproducibility of the analytical techniques, which were confirmed by determining the %RSD of six determinations of analytes at three different concentrations. The %RSD did not surpass 1.82, 1.47, and 1.43% for RST and 1.37, 1.51, and 1.91% for VTN by all three methods, which fall comfortably between the permissible limit. The correctness confirms the correctness of the determination of analytes which was assessed by determining the % retrieval beside the percentage of comparative error. The % recovery of both the analytes in the above three concentration solutions

Table 2: Analysis results of the manually blended solutions

Manually mixed ratio (μg/mL^{-1})	^{0}D and ^{1}D spectra method (% recovery)		RDA (% recovery)		FDR (% recovery)	
RST:VTN	**RST**	**VTN**	**RST**	**VTN**	**RST**	**VTN**
6:25	99.62	98.34	101.25	100.19	100.99	100.73
2:6	101.46	98.47	99.33	99.73	99.46	98.44
5:24	100.86	101.08	98.86	98.55	98.86	99.17
3:15	99.07	101.47	101.09	100.67	100.16	98.75
10:20	98.67	98.64	100.45	100.72	99.43	100.49
Across mean	99.94	99.60	100.20	99.97	99.78	99.52
%RSD	1.18	1.54	1.06	0.89	0.82	1.04

%RSD: %relative standard deviation.

Table 3: Assay results of formulations and statistical comparison

Formulation (mg Tablet^{-1})	^{0}D and ^{1}D spectra (mean % ± SD)		RDA (mean % ± SD)		FDR (mean % ± SD)		Reference method [33]	
RST:VTN	RST	VTN	RST	VTN	RST	VTN	RST	VTN
2:8	99.04 ± 1.72	100.73 ± 0.95	101.16 ± 0.78	98.83 ± 0.82	99.61 ± 0.49	101.09 ± 1.37	100.47 ± 0.82	101.34 ± 1.55
4:16	98.47 ± 0.86	99.62 ± 1.46	101.07 ± 0.91	100.94 ± 1.05	101.15 ± 1.34	99.68 ± 1.19	101.64 ± 1.35	99.18 ± 0.94
df[a]	10	10	10	10	10	10		
Student's *t*-test[b]	0.157	0.907	0.219	1.396	0.881	0.389		
p-value	0.877	0.385	0.830	0.192	0.398	0.704		
F-test[c]	1.929	1.099	1.307	2.400	1.223	1.601		
p-value	0.244	0.460	0.387	0.179	0.415	0.308		

HPLC separation was carried out on C18 RP-HPLC column using acetonitrile: water (pH 4.8) 75%:25%, Flow rate 1.0 ml/min. Wavelength 245 nm.
[a] Degrees of freedom.
[b] *t*-test Critical value 2.228.
[c] *F*-test critical value 5.050 (at $p = 0.05$).

ranged from 98.94–99.95% to 100.36–101.08% for RST and VTN, respectively. Furthermore, the low %RE of 0.86–1.43% and 0.75–1.42% for RST and VTN confirm the correctness of the measurements by the proposed methods.

3.5 Application to analysis of formulation and manual mixed solutions

The acceptable validation results of all three methods suggested the utilization of the offered approaches for the investigation of both analytes from the solid dosage form and manual mixed solutions. The solutions of diverse proportions of RST and VTN were analyzed using all three methods and the percent recovery values ranged between 98.67–101.46% and 98.34–101.47% for RST and VTN, respectively (Table 2). The mean retrieval of analytes from the formulation was between 98.47 and 101.16% and 98.83–101.09% for RST and VTN respectively with low %RE (Table 3). Furthermore, the accurateness of the anticipated techniques was further ascertained by the authentic analyte addition technique. The mean recovery of the added amount of RST and VTN was 99.41–100.09% and 99.62–100.82%, respectively (Table 4). This confirms the lack of intervention of adjuvants in the investigation of analytes. In addition, the compounds under investigation were analyzed using validated reported HPLC methods. The statistical comparison by Students' *t*-test and *F*-test of both methods revealed no difference in the percentage recovery. The computed Students' *t*-test and *F*-test outcomes are much lesser than the critical levels, confirming the lack of statistical dissimilarity in terms of accurateness and precision. These acceptable results allow pharmaceutical industries to utilize the proposed UV-spectrophotometric techniques for concurrent quantification of VTN and RST in pills on a regular basis for quality assurance.

3.6 Evaluation of environmental safety of the methods

The UV spectroscopic method is a green-and-white analytical technique due to its rapid analysis nature and use of

Table 4: Outcomes of authentic drug addition technique

Amount added (μg/mL^{-1})	^{0}D and ^{1}D spectra (% recovery)		RDA (% recovery)		FDR (% recovery)	
RST:VTN	RST	VTN	RST	VTN	RST	VTN
1:4	99.56	99.37	99.12	100.86	98.82	98.46
2:8	101.07	100.79	101.55	99.72	99.06	100.77
3:12	99.64	98.69	98.62	101.88	100.35	99.91
Across mean	100.09	99.62	99.76	100.82	99.41	99.71
%RSD	0.85	1.07	1.57	1.08	0.82	1.17

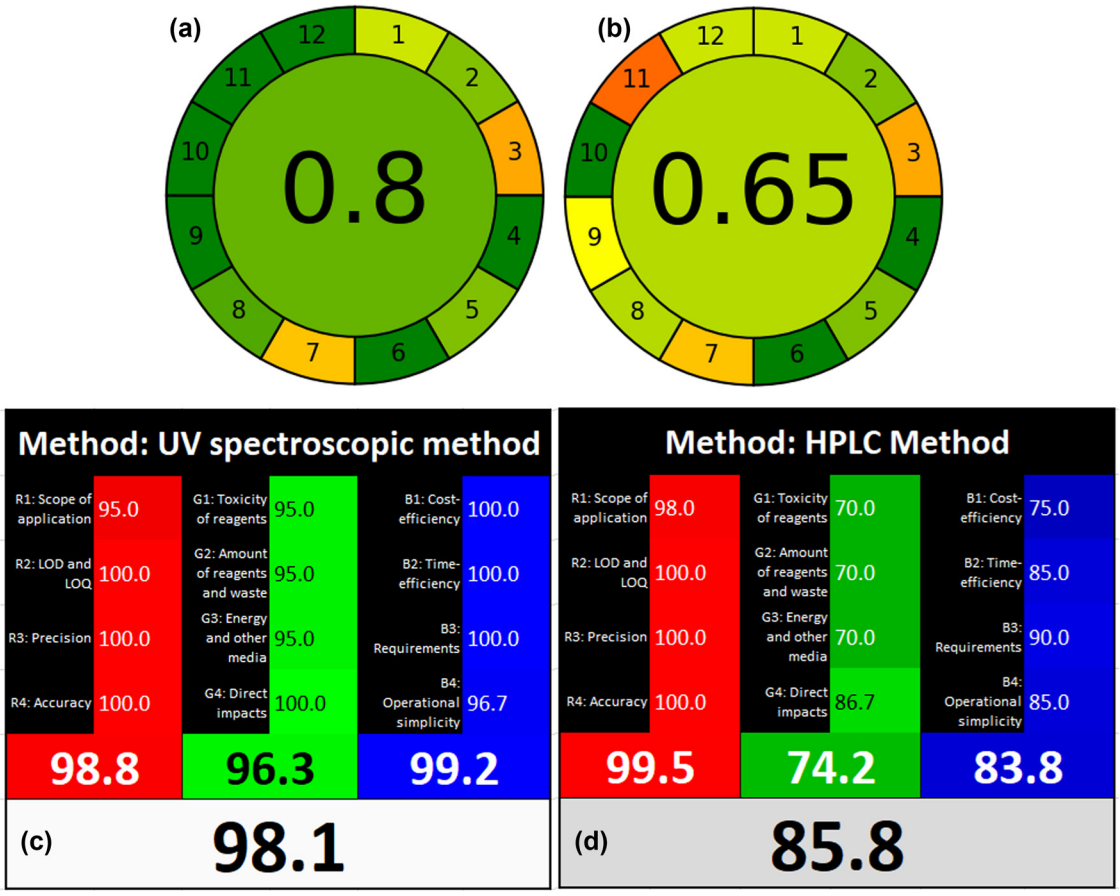

Figure 6: Greenness 6 (a and b) and whiteness (c and d) evaluation results of proposed UV spectroscopic (a and c) and HPLC (b and d) methods.

safe and small quantities of solvents. Further to confirm the greenness and whiteness of the proposed techniques three quantitative eco-friendly nature evaluation tools were applied. The greenness was evaluated using AGREE and whiteness by the GRB method. The UV spectroscopic method pictogram of AGREE evaluation tool showed green with an overall value of 0.8 (Figure 6a), whereas the HPLC method [33] was yellowish green with an overall value of 0.65 (Figure 6b), indicating that the UV spectroscopic method was greener than the HPLC method due to the use of safer solvents, and lesser waste formation during the analysis process. The UV spectroscopic method pictogram of the GRB evaluation tool showed white with an overall value of 98.1 (Figure 6c), whereas HPLC method was gray with an overall value of 85.8 (Figure 6d), indicating that the UV spectroscopic method was safer than the HPLC method. In terms of scope, HPLC is better than UV; however, the safety, ease of operation, waste generation, cost, and time efficiency UV spectroscopic method are better.

4 Conclusions

This study depicted the development of three easy mathematically manipulated UV spectroscopic methods for the estimation of RST and VTN from formulations and manually prepared solutions. These methods involved simple steps of mathematical filtration using the software provided with the instrument, hence economical and rapid. Further, the methods are highly accurate, reproducible, sensitive, and applicable to formulations and laboratory mixed solutions. The evaluation of findings against the stated HPLC techniques confirmed the statistical non-significance in terms of accurateness and precision. Furthermore, the offered approaches follow the green and white analytical principles to make the methods eco-friendly. In summary, the proposed UV spectroscopic techniques were easy, rapid, green, and white analytical techniques for analysis of RST and VTN for regular quantification in formulations.

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