# Smart Spectrophotometric Method Development for Simultaneous Estimation of Antidiabetic Drugs in Formulations

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## ABSTRACT

**Background:** An anti-diabetic formulation consisting of vildagliptin and remogliflozin was prescribed for better glycemic control. In the present study a simple, rapid derivative spectrophotometric methods were evolved to analyze these two analytes from the formulations. **Methods:** Two processed UV spectrophotometric methods were established by measuring the peak amplitude at zero-crossing of second derivative spectra of analytes. The second procedure comprehends the generation of zero - order spectra from the mixture of analyte spectra by division and multiplication by the pure analyte spectra to remove the effect of one of the analytes. **Results:** Both methods showed linearity concentrations in the range of 2-75 $\mu$g/ml for RGF and 2-50 $\mu$g/ml for VGT. The low LOD and LOQ found for RGF and VGT by both methods indicated the good sensitivity of the methods. The mean percentage recovery was 98.60 % and 100.78%, for RGF and 98.81 % and 99.15 % for VGT, with low percent relative error. The % RSD for intra and inter-day precision was less than $\pm 2$%. Finally, the planned methods were employed for the assay of the VGT and RGF from the medicine and the outcomes were matched with the reported methods. **Conclusion:** The assay results of the formulation were in agreement with the concentration of labeled amount and no significant difference was observed in the results when compared to the reported method. Hence, the anticipated procedures could be applied for the routine quality control of formulations consisting of VGT and RGF.

**Key words:** Antidiabetics, Vildagliptin, Remogliflozin, Derivative spectrophotometry, Formulation, Validation.

## INTRODUCTION

The number of patients with diabetes mellitus is increasing rapidly worldwide A recent study by the World Health Organization reported that the occurrence of diabetes mellitus type 2 was more than 30%. Hence, to avoid the diabetic complications monitoring normal blood sugar levels is a key factor in type 2 diabetic patients. In patients with type 2 diabetes, the first line of treatment with metformin did not reduce HbA1c levels below the targeted level, second pharmacological agents like dipeptidyl peptidiase-4 (DDP-4) inhibitors and sodium glucose cotransporter-2 (SGLT-2) inhibitors are prescribed.[1] Several DPP4 inhibitors[2] and SGLT2 inhibitors[3] that offer strong glycemic control have been prescribed alone or along with other antidiabetic drugs. According to a study by Ahsan S[4] vildagliptin in combination with remogliflozin will help to achieve the targeted HbA$_{1c}$ level. Hence, a single tablet consisting of vildagliptin (50 mg) and remogliflozin (100 mg) was sanctioned by FDA because of its simpler regime for better glycemic control. Further both
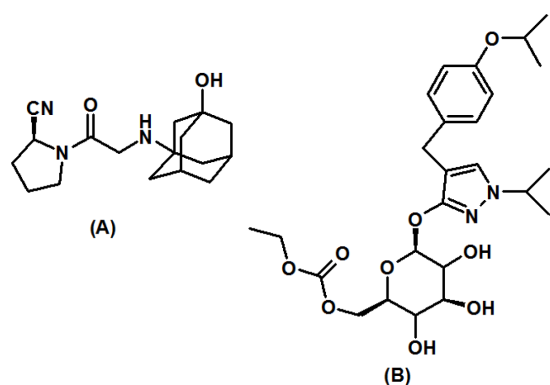
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**Figure 1: Chemical structure of vildagliptin(A) and remogliflozin etabonate (B).**

the analytes act by different mechanism of action and insulin independent. Vildaglitpin (VGT, Figure 1A) a selective, orally active reversible DPP4 inhibitor, acts by inhibiting DPP-4 enzyme responsible for the breakdown of glucagon-like peptide-1 (GLP-1).[2,5] This enhances the secretion of insulin and reduces the secretion of glucagon resulting in a reduction in blood glucose levels. In addition, VGT is well tolerated, does not have any effect on body weight, has no hypoglycemic effect and can be used in patients with kidney problems.[5,6]

Remogliflozin etabonate (RGF, Figure 1B) is the latest SGLT-2 inhibitor, used for the management of type 2 diabetes prescribed alone and with other antidiabetic agents. RGF reduce the blood glucose level by inhibiting the reabsorption of glucose from the renal tubule and independent of effecting the insulin release. In addition, RGF help in declining the body weight and blood-pressure due to increase in the excretion of glucose and water.[2,7]

A number of assay procedures have been described for the assessment of VGT and RGF from different matrix including medicines. VGT has been estimated using UV-Vis spectrophotometry,[8-12] HPLTC,[13] HPLC,[14,15] and LCMS[16] alone and with other antidiabetic agents. The analysis of RGF by spectrophotometry,[17-20] HPLC,[19-22] UPLC,[23] and LC-MS[24] has been reported in the literature. Nevertheless, no quantitative assay method has been described for the concurrent quantification of VGT and RGF from the formulation. Hence, in the present work, we plan to establish simple, sensitive and robust derivative spectrophotometric methods for the quantification of VGT and RGF from combined medicine and validated according to the ICH guidelines.

## MATERIALS AND METHODS

Spectrophotometric measurements were performed using double-beam UV-Vis spectrophotometer (1600, Shimadzu, Japan). The cuvettes used for the measurements were quartz and one cm in width. The instrument was set for fast scanning with 0.1 nm-slit width. The scanned spectra were processed using UV Probe software (Ver. 2.2). The standard drug and tablets were weighed using a Shimadzu digital weighing scale (Japan). Volumetric flasks used for preparation of standard solutions and sample solutions were calibrated before use.

Standard analytes of vildagliptin and remogliflozin etabonate were purchased from Biokemix India Ltd. (Hyderabad, India). Analytical-grade ethanol used for the preparation of standard solution was purchased from Sigma Aldrich. Pure water used for the dilution of standard and sample solutions was prepared using Milli Q water purifier (Millipore, USA).

### Preparation of standard solutions

Standard solutions of VGT and RGF 1 mg / ml were prepared using water and ethanol respectively. Further standard solutions were diluted using water to obtain a concentration in the linearity range.

### Preparation of sample solution

The medicine consisting of VGT and RGF was not available in the local market, hence a physical mixture consisting of VGT, RGF and all tablet excipients was prepared by geometrically adding sufficient amount VGT, RGF, sodium carboxy-methylcellulose, microcrystalline cellulose, anhydrous lactose, and magnesium stearate. Powder equal to 5 mg of VGT and 10 mg RGF was placed into a 10 ml graduated flask and extracted with ethanol to get 0.5 mg/ml of VGT and 1 mg /ml of RGF respectively.

### Procedures

### Second derivative spectrophotometric method (SDS)

Working standard solutions (10 ml) of VGT (2, 10, 20, 30, 40 and 50 μg/ml) and RGF ((5, 15, 30, 45, 60 and 75 μg/ml) were prepared separately by adding sufficient amounts of VGT and RGF solutions respectively. A sufficient amount of ethanol was also added to each volumetric flask to maintain an equal amount (250 μL) of ethanol in each volumetric flask. After that, the absorption spectra were measured for each solution by scanning at a wavelength range of 200-300 nm. Subsequently, the second-order derivative spectra of VGT and RGF were generated from the zero-order spectra using UV-Probe software. The peak amplitudes of the second-order derivative spectra were monitored at 221.3 nm and 243.4 nm for VGT and RGF respectively.

Further, calibration plots were created by plotting the peak amplitudes against analyte concentration and corresponding regression equations were derived.

## Constant centered spectrophotometric method (CCS)

Working standard solutions consisting of 2-50 μg/ml of VGT and 5-75 μg/ml of RGF were organized in 10 ml graduated flasks. After that, the absorption spectra were measured for each solution by scanning at a wavelength range of 200-300 nm. Separately 5 μg/ml of VGT and RGF were prepared and absorption spectra were recorded. The absorption spectra of VGT and RGF were divided by the spectrum of 5 μg/ml of VGT and RGF separately to obtain the ratio spectra of RGF and VGT respectively. The resulting ratio spectra of VGT and RGF were multiplied with 5 μg/ml of RGF and VGT spectra to generate the original spectra of VGT and RGF correspondingly. The absorption was calculated at 208 nm and 225.8 nm for VGT and RGF correspondingly. The calibration curves (Figure 2c and 2d) were generated by plotting the absorption against the analyte concentration and regression equations were computed.

## Application to medical dosage forms

Above prepared formulation, solutions were diluted with water to obtain the concentrations of VGT and RGF in the linear range. The UV absorption spectra were measured by scanning at a wavelengths between 200-300 nm. Subsequently, the spectra were processed into second –order derivative spectra and the peak amplitudes were measured at 243.4 nm for RGF and 221.3 nm for VGT. The quantity of analytes was computed using the respective regression equations. Further, for the CCS method, the zero-order spectra of VGT and RGF were generated using the CCS method described above. The peak absorption was determined at 208 nm and 225.8 nm for VGT and RGF correspondingly and the amount of analytes was determined from the corresponding regression equations.

## RESULTS AND DISCUSSION

In general, derivative spectrophotometric methods have been applied for the quantification of mixtures of compounds with overlapping spectra.[25-30] The derivatization technique is a sensitive, rapid and accurate technique for the concurrent determination of multi-component formulations. Derivatization removes the absorption of one of the components at the zero-crossing point and allows the quantification of another component.[25,26] In the present work, RGF and VGT

showed complete overlap (Figure 2A); hence, they were changed into 2nd derivative spectra using a scaling factor of 100 (Figure 2B). Different wavelengths from 2 nm to 8 nm were envisaged as Δλ; however, 4nm showed smooth spectra, hence, 4 nm was selected as Δλ. VGT showed zero crossing points at 208.2 nm, 243.4 nm, where RGF had some absorption, however, at the 243.3 nm selectivity was better hence, 243.3 nm was selected for further study. RGF showed two zero crossing points at 235.1 nm and 221.3 nm where VGT had some absorption. However, at 221.3 nm the peak amplitude and selectivity were better, hence 221.3 was selected for further study. Standard UV absorption spectra of VGT and RGF were converted in to second derivative spectra (Figure 3A and 3B). Farther, the peak amplitude of second derivative spectra of pure and mixture showed same amplitude at 221.3 nm for VGT (Figure 2B) and at 243.3 nm for RGF (Figure 2B).

Constant center spectrophotometric method is another simple technique used for simultaneous quantification of analytes present in mixture. In this method, for the quantification of RGF, UV absorption spectra were recorded for the mixture of analytes (VGT+RGF) and divided by the spectrum of one of the analytes (5 μg/ ml UV spectrum of VGT). The obtained ratio
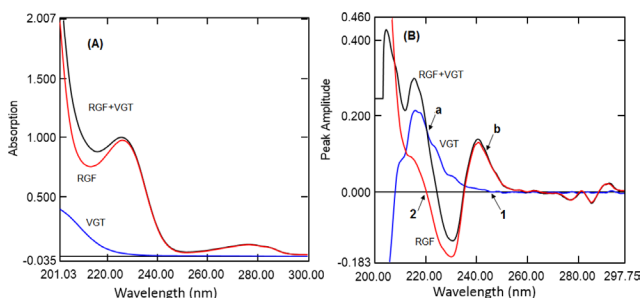


**Figure 2: Zero-order spectra of VGT, RGF and mixture (A). Second derivative spectra of VGT, RGF and mixture showing zero crossing points of VGT (1) and RGF (2) along with same absorption for VGT (a) and RGF (b) form pure and mixture spectra.**
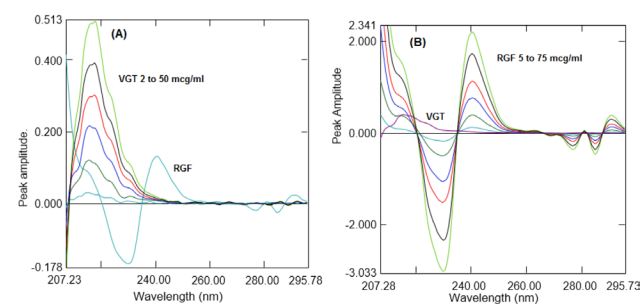


**Figure 3: second order derivative spectra of VGT (2 to 50 μg/ml) (A) and RGF (5 to 75 μg/ml) Constant center spectrophotometric method.**
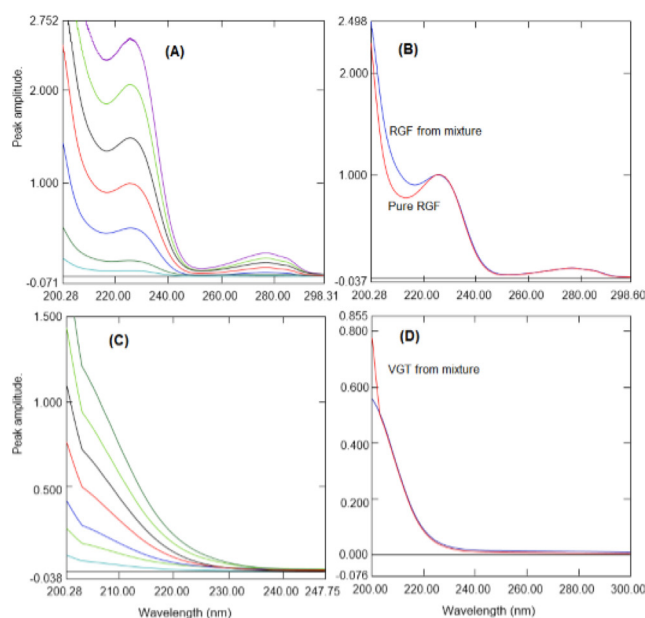
**Figure 4: Zero-order spectra of RGF (2 to 75 μg/ml) developed by dividing the mixture by the spectrum of VGT (5 μg/ml) followed by multiplication with the spectrum of VGT (A). Zero-order spectra of VGT (2 to 50 μg/ml) were developed by dividing the mixture by the spectrum of RGF (5 μg/ml) followed by multiplication with the spectrum of RGF(C). Zero-order spectra generated from mixture and pure RGF (B) and VGT (D).**
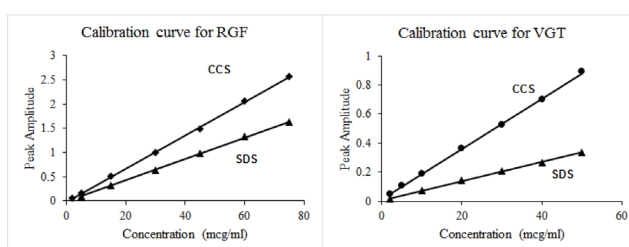


**Figure 5: Calibration curve for RGF(A) and VTG(B) by both the second derivative spectroscopic (SDS) and constant-centered spectroscopic (CCS) methods.**

spectra illustrate the RGF/VGT+ constant. The zero-order spectra of RGF (Figure 4A) can be obtained by multiplying the resulting ratio spectra with the same VGT spectra. The calibration plot was generated by measuring the peak amplitude at 225.8 nm against corresponding concentration of RGF (2, 5, 15, 30, 45, 60 and 75 μg/ ml) and regression equations were computed. The spectrum generated from the mixture and pure analytes showed the same absorption at 225.8 nm (Figure 4B).

Similarly, VGT zero-order spectra (Figure 4C) were generated by dividing the mixture spectra with the RGF spectrum, followed by multiplication of the ratio spectra with the RGF spectrum (5 μg/ ml UV spectrum of RGF). Calibration plots and regression equations were generated using peak amplitude at 208.0 nm and corresponding concentrations (2, 5, 10, 20, 30, 40 and 50 μg/ ml). Further, the zero-order spectra generated from the mixture and pure VGT showed the same absorption at 20.0 nm (Figure 4D).

### Method validation

Validity of the proposed derivative methods were confirmed by performing linearity, limit of detection, limit of quantification, accuracy, precision and stability studies as per the ICH guidelines.

### Linearity

The calibration plot provides important information about the linearity of the proposed method. In the present study linearity range was studied in the concentration range of 5-75 μg/ml using the SDS method and 2-75 μg/ml by the CCS method for RGF (Figure 5A). VGT exhibited excellent linearity in the range of 2 to 50 μg/ml by both the methods (Figure 5B). The regression equations and regression coefficients are listed in Table 1.

### Limit of detection and quantification limits

The detection and quantification limits were estimated using the linearity curve parameters. The LOD was calculated as 3.3 times the standard deviation of the intercept to the slope of the curve. The LOQ was 10 times the standard deviation of the intercept to the slope of the curve. The low LOD and LOQ values are tabulated in Table 1, indicating the good sensitivity of the proposed methods.

### Precision

The precision of the established procedures was also assessed in terms of intra and inter-day by analyzing three concentrations of both analytes in the calibration curve range. For intra-day solutions were analyzed tree times in day and these solutions were investigated for three succeeding days for inter day precision. The percent relative standard deviation was calculated and presented in Table 1. The result showed low percent RSD, which confirmed that the proposed methods were precise.

### Accuracy

Accuracy of the projected procedures were examined by assaying different concentration of both the analytes in the calibration concentration range. The accuracy of the methods was expressed in terms of the percent recovery and percent relative error. The mean percentage recovery was 98. 60 % to 100.78%, for RGF and 98.81 % to 99.15

**Table 1: Validation parameter results of the proposed spectroscopic methods for simultaneous determination of VGT and RGF.**

| Validation Parameters | Remogliflozin | | Vildagliptin | |
|---|---|---|---|---|
| | SDS | CCS | SDS | CCS |
| Wavelength (nm) | 243.4 | 225.8 | 221.3 | 208.0 |
| Linearity range (µg/mL) | 5 - 75 | 2 - 75 | 2 - 50 | 2 - 50 |
| Slope | 0.022 | 0.0342 | 0.0066 | 0.0173 |
| Intercept | -0.0141 | -0.012 | 0.0064 | 0.0148 |
| Regression coefficient ($r^2$) | 0.9997 | 0.9995 | 0.9993 | 0.9996 |
| LOD (µg/mL) | 1.38 | 0.25 | 0.31 | 0.29 |
| LOQ (µg/mL) | 4.12 | 0.76 | 0.94 | 0.89 |
| Accuracy (Mean %±%RE) | 98.60±0.890 | 100.78±1.426 | 98.81±1.482 | 99.15±0.849 |
| Precision (%RSD) | | | | |
| Intra day | 1.245 | 0.943 | 0.905 | 1.098 |
| Inter day | 0.852 | 1.846 | 1.067 | 0.804 |

SDS: Second Derivative Spectrophotometric method; CCS Constant Centered Spectrophotometric method; %RE: Percentage Relative Error

**Table 2: Assay and statistical calculation results.**

| | Amount taken (µg/mL) | Second Derivative method | Constant Centered method | Reference Method |
|---|---|---|---|---|
| | | % Recovery | % Recovery | % Recovery |
| Vildagliptin | | | | |
| Formulation (50 mg VGT +100 mg RGF) | 8 | 99.45 | 98.25 | 98.00 |
| | 16 | 98.54 | 99.25 | 99.50 |
| | 32 | 101.36 | 100.56 | 98.89 |
| | Mean | 99.78 | 99.35 | 98.79 |
| | %RSD | 1.523 | 1.156 | 0.754 |
| | n | 4 | 4 | |
| Student *t*- test (2.776)[c] | | 1.009 | 0.348 | |
| *F (19)d* | | 4.077 | 1.18 | |
| Remogliflozin | | | | |
| Formulation (50 mg VGT +100 mg RGF) | 16 | 101.20 | 99.20 | 99.03 |
| | 32 | 100.75 | 100.74 | 100.90 |
| | 64 | 99.00 | 98.59 | 99.72 |
| | Mean | 100.31 | 99.51 | 99.88 |
| | %RSD | 1.162 | 1.114 | 0.957 |
| | n | 4 | 4 | |
| Student *t*- test (2.776)[c] | | 0.363 | 0.436 | |
| *F (19)d* | | 1.411 | 1.353 | |

[a] HPLC method using monolithic column (5 µm, 50 mm × 4.6 mm i.d.), and mobile phase acetonitrile-sodium dihydrogen phosphate (10 mM) and SDS (10 mM) (30/70, v/v) with pH 4.5, flow rate of 2.5 mL/min.[31]
[b] HPLC method using monolithic column (5 µm, 50 mm × 4.6 mm i.d.), and mobile phase acetonitrile and mixture of 25 mM sodium dodecyl sulfate and 10 mM potassium dihydrogen phosphate (pH 3.5) (42:58 *v/v*).[19]
[c] and [d] are critical values of *t* and *F*, respectively at *p*=0.05

% for VGT with low % RE, confirmed the accuracy of the projected methods (Table 1).

## Stability Studies

Stock solutions of RGF and VGT were prepared and stored in a refrigerator at 4°C. Working standards and standard solutions for method validation were prepared on daily basis. The stability of the stock and sample solutions was evaluated by analyzing the solutions stored in the refrigerator after being kept at room temperature for 5 h and after 7 days. The assay results of freshly prepared solutions and the stored solutions did not

show significant differences, indicating the stability of the solutions.

## Application to medicinal formulations

The proposed procedures were applied for quantitative analysis of VGT and RGF from the medicine. This amount was in agreement with the labeled quantity. The assay results confirmed that the excipients did not affect the assay performance. This indicates the specificity of the suggested approaches. Further, the assay outcomes were compared with the reported HPLC methods[19,31] using the *F*-test and students *t*-test. The results indicated that there were no significant differences between the performance of the suggested and the described HPLC methods regarding precision and accuracy as the critical values of *F* and *t*-tests were higher than the calculated values (Table 2).

## CONCLUSION

In summary, a rapid and sensitive second-order derivative and constant centered spectrophotometric methods were established for the concurrent assay of VGT and RGF for the first time. The process was fully validated following the ICH recommendations. Further, the derivative spectrophotometric methods were applied to assess VGT and RGF in formulations with a high percentage recovery; of more than 98%. The assay results of the formulation were in agreement with the concentration of labeled amount and no significant difference was observed in the results when compared to the reported method. Hence, the anticipated procedures could be applied for the routine quality control of formulations consisting of VGT and RGF.

## ACKNOWLEDGEMENT

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**UV:** Ultra violet; **%RE:** Percentage Relative Error; **%RSD:** Percent Relative Standard Deviation; International Conference on Harmonisation; **DDP-4:** dipeptidyl peptidase-4; **SGLT-2:** sodium glucose cotransporter-2; **HPLC:** High Performance Liquid Chromatography; **UPLC:** Ultra Performance Liquid Chromatography; **LCMS:** Liquid Chromatography Mass spectrometry.
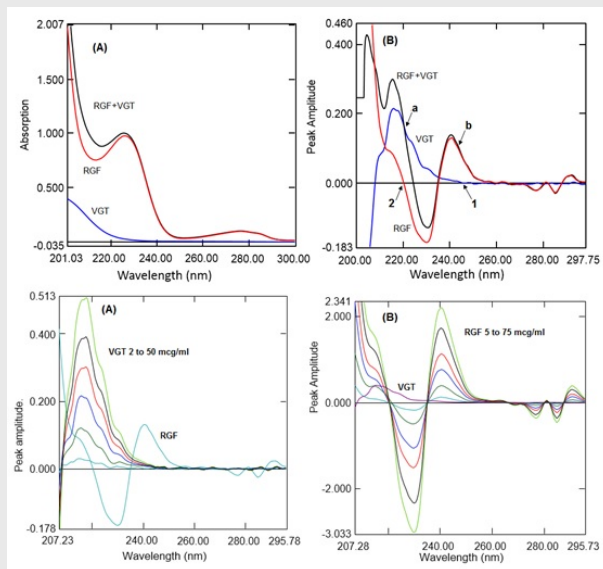
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## SUMMARY

Recently approved fixed dose combination of vildagliptin and remogliflozin etabonate are useful for the treatment of the type 2 diabetes. A simple, rapid, accurate derivative UV spectroscopic methods were established for the concurrent quantitative analysis of both the analytes. Both methods showed linearity concentrations in the range of 2-75 µg/ml for RGF and 2-50 µg/ml for VGT. The low LOD and LOQ found for RGF and VGT by both methods indicated the good sensitivity of the methods. The good percentage recovery along with low percent relative error confirmed the accuracy of the methods. The % RSD for intra and inter-day precision was less than ±2%. The assay results of the formulation were in agreement with the concentration of labeled amount and no significant difference was observed in the results when compared to the reported method. Hence, the anticipated procedures could be applied for the routine quality control of formulations consisting of VGT and RGF.

## PICTORIAL ABSTRACT



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