Original Article

Smart UV Derivative Spectrophotometric Methods for Simultaneous Determination of Metformin and Remogliflozin: Development, Validation and Application to the Formulation

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ABSTRACT

Background: The result of pharmaceutical industry research for the new class and the new combination of drugs for the treatments of diabetes is the newly approved combination of metformin (MET) and remogliflozin (REM). For the quality control of this formulation, three smart, reproducible and non-sophisticated spectroscopic techniques were developed by modification of UV spectra. **Materials and Methods:** The first two methods were based on the measurements of the peak height of the third derivative and second derivative ratio spectra of MET and REM and the third method was the constant center spectrum subtraction method. **Results:** The proposed methods exhibited Beer's law in the range of 2.5 to 30 μg/ml and 1 to 24 μg/ml for MET and REM correspondingly by all three methods. The mean percentage recovery was found to be in the range of 99.08% to 100.15% for MET and 98.73% to 100.27% for REM. Further, both analytes were quantified from the formulation using proposed spectroscopic methods with high accuracy. Comparison of all three methods with the reported HPLC method showed no variation in the assay outcomes in relation to accuracy and precision. **Conclusion:** The suggested techniques are simple, accurate and reproducible, hence could be used for regular quality control of formulation consisting of MET and REM.

Key words: Metformin, Remogliflozin, UV, Derivative spectroscopy, Validation, Formulation.

INTRODUCTION

Metformin HCl, chemically known as 1,1-Dimethylbiguanide hydrochloride (Figure 1A) is extensively used for the management of diabetes mellitus Type-2. It is a first choice for the management of blood glucose due to its multiple mechanisms of action and safety.[1-3] However, slowly glycemic control reduces hence patients require a higher dose of metformin or additional anti-diabetic drug for reducing the blood glucose level to normal.[3] Sodium-glucose transport protein (SGLT) inhibitors are recent among the many new class of antidiabetic drugs developed during the past two decades. Inhibition of SGLT rises the elimination of glucose by inhibiting the reabsorption of glucose from the glomerular filtrate.[4,5] Several SGLT inhibitors such as canagliflozin, empagliflozin dapagliflozin are successfully administered with metformin.[6] Recently, Food and Drug Administration has approved a combination of remogliflozin etabonate (Figure 1B) and metformin film-coated tablets in two strengths of 500 mg / 1000 mg MET with 100 mg REM.[7] REM chemically known as β-D-Glucopyranoside,

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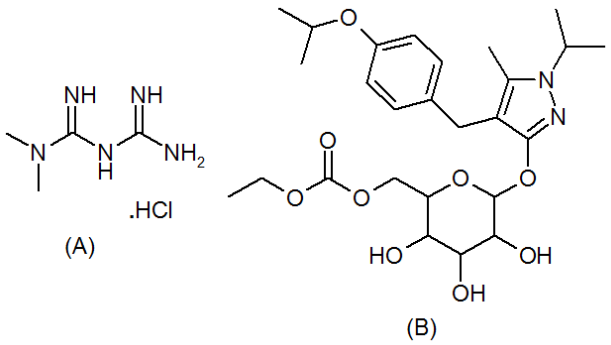


Figure 1: Chemical Structures of Metformin HCl (A) and Remogliflozin etabonate (B)

5-methyl-4-[[4-(1-methylethoxy)phenyl]methyl]-1-(1-methylethyl)-1H-pyrazol-3-yl, 6-(ethyl carbonate), acts by inhibiting specific enzyme SGLT-2 and is insulin-independent anti-diabetic drug. In addition, REM helps in reducing the body weight and blood pressure in diabetes mellitus patients.[8-10]

Many analytical methods were illustrated for the estimation of MET from pharmaceutical preparations and plasma by spectroscopic,[11,12] HPLC,[13-15] HPLTC,[16] LC-MS,[17,18] and CZE,[19,20] alone and with other drugs. Few analytical procedures were also depicted in the texts for the analysis of REM.[21-22] UV spectroscopic and HPTLC procedures were reported for the quantification of REM in the solid dosage forms,[21] whereas LC-MS/MS procedure has been utilized for analysis of REM in the human plasma.[22] Two stability-indicating liquid chromatographic procedures for the assay of REM from the bulk and formulations were also reported in the literature.[23,24] Recently, Tammisetty *et al*.[25] reported UPLC method and our team has reported RP-HPLC and derivative spectroscopic procedures[26,27] for the concurrent determination of MET and REM from formulations. The reported UV derivative spectroscopic methods were based on first and 2nd derivative spectroscopic methods, however, the use of higher derivative is preferred for better resolution of interfering analytes, hence in the present work third derivative spectroscopic method was developed. Further, in continuation of our research work on the development of a simple spectroscopic method,[28,29] three smart and simple UV derivative spectroscopic techniques were reported for the concurrent analysis of MET and REM from formulations.

MATERIALS AND METHODS

Chemicals and instrument:

A dual beam UV spectrophotometer (Shimadzu UV-Vis 1700, Tokyo, Japan) was used for recording the UV spectra of solutions by 10 mm quartz cuvettes using ethanol-water as a solvent. The instrument was adjusted with 2 nm slit width and medium scanning speed. Recorded spectra were manipulated using UV-probe (Ver. 2.2) software provided with the instrument. To generate smooth manipulated spectra, scanned spectra were smoothened by using 4 nm wavelength. Active pharmaceutical drugs metformin hydrochloride (purity 98.96%) and remogliflozin etabonate (purity 99.2%) were purchased from Bioteck India Limited, Hyderabad, India. Ethanol secured from Sigma Aldrich was an analytical grade. Milli Q (Millipore, USA) was used to prepare the ultra-pure water.

Standard stock and working solutions

The 25 mg of MET and REM were dissolved separately into two 25 ml calibrated flasks containing 15 ml of water and ethanol respectively. Analytes were dissolved by mixing with sonicator for 10 min and the final volume was adjusted with respective solvents to get standard stock solutions (1 mg/ml) of MET and REM. Further, stock solutions were converted into working standard solutions of 100 µg/ml by adding the required amount of water to the aliquot of stock solution.

Preparation sample solutions

The marketed MET and REM tablets were not accessible in the resident market, hence simulated tablet powder was arranged by adding the required amount of MET, REM and tablet adjuvants (Talc, Hydroxypropyl methylcellulose, magnesium stearate, Primogel, sodium starch glycolate) to get a mixture of MET and REM in a ratio 500:100 and 1000:100. The mixture corresponding to 100 mg of REM, with 500 mg and 1000 mg of MET was transferred separately into 100 ml graduated flasks and solubilized in 50 ml ethanol. Clear solutions were prepared by swirling the solutions for 10 min and volume was completed with water. Additionally, to bring the amount of both analytes in the linearity range, a sufficient amount of water was added to the aliquot of the sample solution.

Procedure

Third derivative spectroscopic method (TDS)

For the construction of calibration curve, seven solutions in the concentration of 2.5, 5,10, 15, 20, 25, 30 µg/ml of MET and 1, 4, 8, 12, 16, 20, 24 µg/ml of REM were arranged by diluting appropriate volume of working standard solutions separately. UV absorption spectra were recorded for these solutions in the range of 200 nm to 300 nm and were saved in the computer. Further, zero-order spectra were changed into 3rd derivative spectra utilizing 4 nm as Δλ with ascending factor 100.

Then the peak amplitude was determined at 240.1 nm for MET and a linear curve was generated by plotting a graph against the respective concentration. Similarly, a linear curve was prepared for REM by determining the peak height at 234.8 nm. Regression equations were also generated along with the regression coefficients.

Ratio Second Derivative Spectroscopic method (RSDS)

Seven aliquots of MET and REM working standard solutions were transferred into 10 ml calibrated flasks, to obtain the amount of MET in the series of 2.5 to 30 µg/ml (2.5, 5, 10, 15, 20, 25, 30) and REM in the range of 1 to 24 µg/ml (1, 4, 8, 12, 16, 20, 24 µg/ml). UV absorption spectra were documented for these solutions in the range of 200 nm to 300 nm against dilute ethanol as solvent. Separately, MET and REM solutions comprising of 10 µg/ml and 20 µg/ml respectively were prepared and scanned. For measurement of MET, ratio spectra of MET were generated by dividing the saved combined spectra of REM and MET by the UV spectrum of REM. Further, these spectra were changed into second-order derivative spectra employing 4nm as Δλ using an ascending factor of 10. Then the linearity curve was generated by determining the peak height at 246.6 nm and plotting the graph against the respective concentration of MET. Similarly, for the measurement of REM, ratio spectra of REM were generated by dividing the REM and MET mixture spectra by the UV spectrum of MET followed by conversion to second derivative spectra. Latter peak height was determined at 277.2 nm and plotted against the respective concentration of REM. Also, regression equations were generated from both the linearity curves and used for the calculations of the concentration of sample solutions.

Constant Center-Spectrum Subtraction method (CCSS)

Using the above-recorded ratio spectra of REM the peak height was determined at 248.6 nm and 277.8 nm and the difference was calculated. Further, peak height was recorded at 277.8 nm from the same ratio spectra and the linear graph was plotted between these peak amplitude values to generate a regression equation to identify the constant value. Similarly, from the ratio spectra of MET peak height difference was determined by deducting the peak height at 221.2 nm from 247.4 nm. Then linear graph was plotted against peak amplitude at 247.4 nm and a calibration curve was generated. The constant value was deducted from the ratio spectra of REM individually followed by multiplication with UV spectra of 10 µg/ml MET spectra to develop zero-order spectrum of REM. Then the absorption was measured at λ_{max} 226.2 nm and a linearity curve was created. Similarly, zero-order spectra of MET were computed and the calibration curve was constructed.

Application of proposed methods to the formulation

The required amount of sample solution comprising 100 mg REM with 500 mg /1000 mg of MET was diluted separately to get the concentration in the range of linearity range. Then the UV spectra were recorded and saved. For the TDS method, the saved zero-order spectra were converted into 3rd order derivative spectra by applying 4nm as Δλ with ascending factor 100 and peak amplitude was determined at 234.1 nm for REM and 240.1 nm for MET. For the RSDS method, the above spectra were divided by the UV spectrum of REM (20 µg/ml) and MET (10 µg/ml) separately, to generate ratio spectra of MET and REM respectively. Then these spectra were converted into second derivative spectra using 4nm as Δλ with a scaling factor of 10. Peak amplitude at 277.2 nm was measured for REM and at 246.6 nm for MET. Further, the constant value was deducted from the respective ratio spectra and the resulted spectra were multiplied by UV spectrum of REM (20 µg/ml) and MET (10 µg/ml) separately to generate zero spectra of MET and REM respectively. Absorption was measured at 226.2 nm from the REM spectrum and 232.9 nm from the MET spectrum. Finally, concentration was calculated using the respective regression equations.

RESULTS AND DISCUSSION

UV spectrophotometry is a simple, rapid and reproducible analytical technique used extensively for the quantification of drugs from the formulations. However, quantification of multi-component formulation having overlapping spectra is difficult. Hence, different manipulation of UV spectra has been utilized to analyze the analytes in multicomponent formulations without prior separation.[28-31] In the present study, the over led

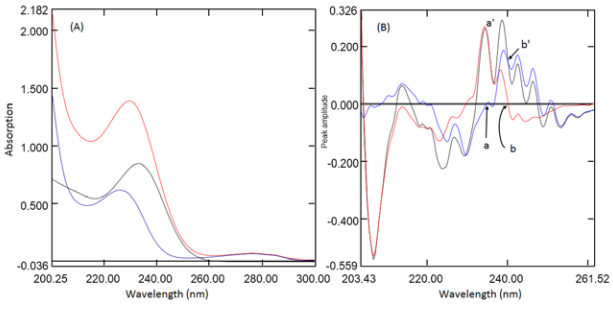


Figure 2: Normal UV spectra of MET, REM and Mixture (A); Third derivative UV spectra of MET, REM and Mixture (B); points "a" and "b" are zero crossings for MET and REM respectively; points a' and b' showing same peak amplitude in pure and mixture.

UV spectra (Figure 2A) of MET and REM revealed total overlap throughout wavelength of 200 nm to 300 nm. Hence, derivatization of UV spectra technique was adopted for concurrent analysis of MET and REM from pharmaceutical preparation. Both analytes did not show any absorbance in the range of 300 nm - 400 nm, hence, the analysis was carried out only in the range of 200 nm – 300 nm.

Third derivative spectroscopic method (TDA)

Derivatization of UV spectra method is a simple, perfect and reproducible technique for concurrent determination of multicomponent formulations without prior separation. Further, the use of higher derivatives provides better resolution of overlapping spectra, hence a third derivative spectroscopic was developed. The basic principle involved is a determination of peak height of derivative spectra of one of the components at the zero-crossing point, where the second analyte as well as formulation excipients, will have zero absorbance. Measuring only one analyte without interference from other analytes and excipients. In the present work, zero-order spectra of REM and MET were converted into third-order derivatives (Figure 2B). Different wavelengths of 2, 4, 8 nm were studied as Δλ, nevertheless, 4 nm exhibited superior reproducibility with smooth spectra. REM showed two zero crossings at 292.2 nm, 240.1 nm and 257.5 nm, at which MET had good absorption, however at 240.1 nm MET showed high absorption and less intercept. Whereas MET spectra had 4 zero crossing points at 208.0nm, 219.8, 234.8 and 248.2 nm, however at 208 nm and 219.8 nm the linearity range was low, whereas at 248.2 the intensity of absorption was low. Further, at 234.8 nm REM showed good peak amplitude and good correlation. Hence 240.1 nm and 234.8 nm were chosen for the further analysis of MET and REM correspondingly. (Figure 3). Further, the third derivative spectra of individual analytes and a mixture of analytes containing the same amount of analyte showed similar peak amplitude. (Figure 2B)

Ratio Second Derivative Spectroscopic method (RSDS)

The ratio derivative spectra method has been used to analyze both components from the binary mixture without prior separation. The basic principle of ratio derivative spectroscopic method involves division of UV spectra of a mixture of analytes metformin (M) and remogliflozin (R) (M + R) with UV spectra of the appropriate concentration of one of the component (R') to generate a ratio spectra. (Figure 4)[27] The newly generated ratio spectra of components are presented as equation 1

$$(M+R)/R' = M/R'+R/R' \quad (1)$$

Where M is metformin and R is remogliflozin, R' remogliflozin with divisor concentration.

In equation 1, R/R' is constant, this constant can be excluded by transforming the ratio spectra into derivative spectra which showed many maximum and minimum. The peak amplitudes at maximum and minimum are directly proportional to concentration. Further, derivatization removes the effect of other analytes and formulation measuring only one analyte at zero-crossing wavelength of another analyte. In the present work, UV spectra of mixture of MET (2.5, 5, 10, 15, 20, 25 and 30 µg/ml) and REM (1, 4, 8, 12, 16, 20 and 24 µg/ml) was divided with spectrum of REM (20 µg/ml) for measurement of MET. Different concentration spectra of REM were tried, however, 20 µg/ml showed better recovery. Similarly, for measurement of REM, mixture spectra with increased concentration were divided with MET (10 µg/ml) spectrum. Then the ratio spectra were changed into second derivative spectra with a scaling factor of 10. Different wavelengths of 2 nm, 4 nm and 8 nm were envisaged as Δλ, nevertheless, 4 nm displayed better accuracy and smooth spectra. The second derivative

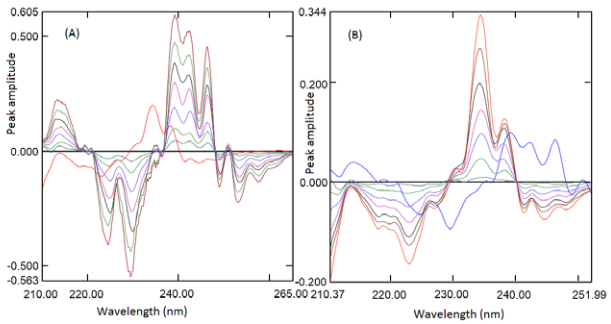


Figure 3: Third derivative UV spectra of MET (2.5, 5, 10, 15, 20, 25 and 30 µg/ml with REM (A); Third derivative UV spectra of REM (1, 4, 8, 12, 16, 20 and 24 µg/ml with MET (B).

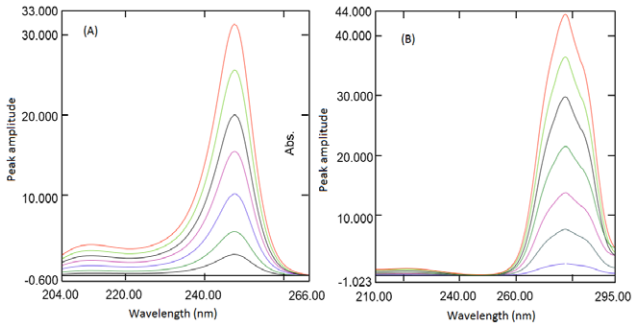


Figure 4: Ratio spectra of MET (2.5, 5, 10, 15, 20, 25 and 30 µg/ml) using 20 µg/ml REM spectrum (A); ratio spectra of REM (1, 4, 8, 12, 16, 20 and 24 µg/ml) using 10 µg/ml MET spectrum(B).

spectra of REM displayed two maximum at 263.2 nm and 292.1 nm and three minimum at 270.3 nm, 277.2 nm and 285.5 nm. The 2nd derivative spectra of MET exhibited two maximum and one minimum at 237.9 nm, 253.4 nm and 246.6 nm respectively. However, for REM 277.2 nm and MET 246.6 nm were selected for the quantification, (Figure 5A) because these are zero crossings for one analyte where another analyte had good absorption. Further, ratio second derivative spectra of a mixture of both analytes and spectra of individual pure analytes consisting of the same amount of analytes showed similar peak amplitude (Figure 5B).

Constant centered-Spectrum subtraction method (CCSS)

The constant centered subtraction method involves the conversion of spectra of a mixture of two analytes into individual zero-order spectra and measurement of absorption at their λ_{max} wavelength. This can be achieved by the following steps, namely determination of constant by amplitude difference and its subtraction followed by multiplication. For the present study, from the above-obtained ratio spectra, peak amplitude was computed at two different wavelengths (λ1 and λ2), subtraction of peak amplitude from one another will cancel the constant (R/R') according to the below equation 2;

$$P_1 - P_2 = (M/R')_1 + Constant - [(M/R')_2 + Constant] \quad (2)$$

Which can be further simplified to

$$P_1 - P_2 = (M/R')_1 - (M/R')_2$$

Where P_1 and P_2 are peak amplitude of spectra at λ_1 and λ_2 correspondingly.

In the present work, the two wavelengths selected were 248.6 nm and 277.8 nm for REM; 221.2 nm and 247.4 nm for MET.

Further, a linearity curve was built by plotting a graph between the peak height difference of ratio spectra of M against peak height of ratio spectra at one of the selected wavelengths (277.8 nm for REM and 247.4 nm for MET). The generated regression equation (3) can be used to calculate the hypothesized $(M/R')_1$, hence

$$(M/R')1-(M/R')2 = slope(M/R')1+intercept \quad (3)$$

$$\Delta P = slope\ P_{hypothesized} + intercept$$

$$\Delta P_{MET} = 1.144x - 0.0333 \text{ (Supplementary file S1)}$$

$$\Delta P_{REM} = 1.1151x - 0.0793 \text{ (Supplementary file S1)}$$

Further, the constant R/R' can be computed by subtracting the hypothesized P value from the noted peak height of mixture at the identical wavelength, this represents only R/R' as per the below equation (4)

$$R/R' = [M/R'+R/R']_1 - (M/R')_1 \quad (4)$$

$$C.V = P_{measured} - P_{hypothesized}$$

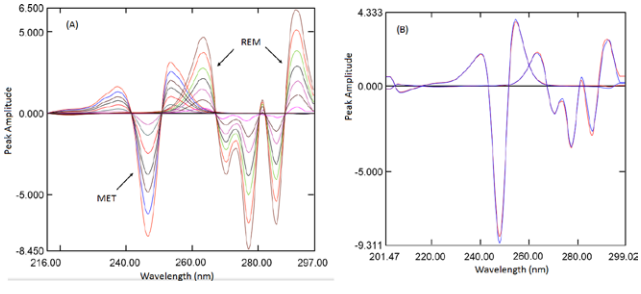
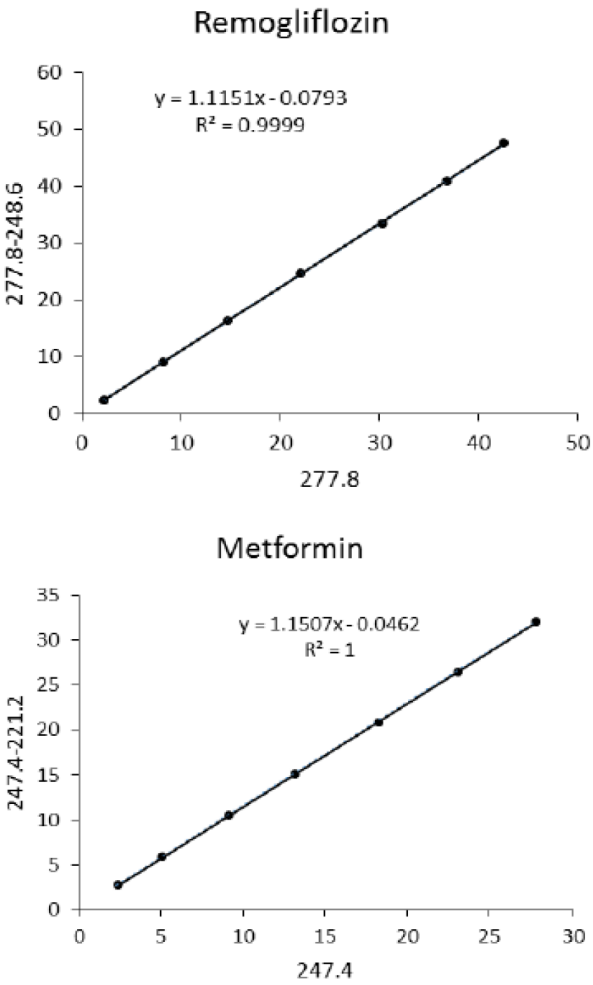


Figure 5: Ratio second derivative spectra of MET (2.5, 5, 10, 15, 20, 25 and 30 µg/ml) Ratio second derivative spectra of REM (1, 4, 8, 12, 16, 20 and 24 µg/ml) (A) and Ratio second derivative spectra of pure and mixture showing same peak amplitude (B).



Supplementary file S1: Calibration curves for MET and REM developed by a peak height difference of ratio spectra against peak height of ratio spectra at one of the selected wavelengths.

Where C.V is R/R' (constant value), $P_{measured}$ is the peak amplitude determined from the ratio spectra of the mixture at 221.2 nm and $P_{hypothesized}$ is calculated amplitude value from the specified regression equation at the same wavelength.

Further, the obtained constant value (R/R') was subtracted from the ratio spectra as per equation 5, followed by multiplication with the spectrum of R (equation 6) used for creating the ratio spectra, which will give zero-order spectra of M (Equation 7, Figure 6A). Further, the regression equation was extracted from the linearity curve plotted by measuring the absorbance of the zero-order spectrum of M at its λ_{max} (232.9 nm) against the corresponding concentration of M.

$$(M+R)/R' = M/R'+R/R' - R/R' \quad (5)$$

$$(M+R)/R' = M/R' * R' \quad (6)$$

$$(M+R)/R' = M \quad (7)$$

Similarly, zero-order spectra of R (Figure 6B) was generated, by subtracting the constant value followed by multiplication with the spectrum of M used for creating the ratio spectra of R and calibration curve was constructed by mearing absorbance at its λ_{max} (226.2 nm) against the corresponding concentration of R. Further, ratio spectra (Figure 7A) and zero-order spectra (Figure 7B) generated from a mixture of analytes and the pure analytes consisting of same amount analytes showed similar absorbance (Figure 7).

Method validation

The proposed UV derivative spectroscopic techniques were validated for various ICH parameters such as linearity range, the limit of detection and limit of quantification, accuracy, precision and stability.[32]

Linearity, LOD and LOQ

Seven solutions of MET (2.5, 5, 10, 15, 20, 25, 30 µg/ml) and REM (1, 4, 8, 12, 16, 20 and 24 µg/ml) were analyzed in triplicate. MET showed linearity in the range of 2.5 to 30 µg/ml and REM in the range of 1 to 24 µg/ml with an excellent correlation coefficient (r^2 > 0.998) by all three methods. (Supplementary file S2) The experiments were performed as per the above-mentioned procedure, then the calibration curves were

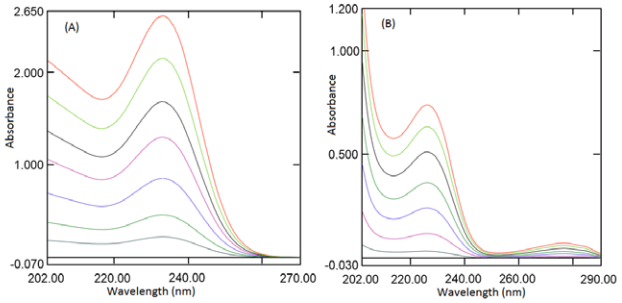


Figure 6: Zero order spectra of MET (2.5, 5, 10, 15, 20, 25 and 30 µg/ml) generated after multiplication with 20 µg/ml REM spectrum (A); zero order spectra of REM (1, 4, 8, 12, 16, 20 and 24 µg/ml) generated after multiplication 10 µg/ml MET spectrum(B).

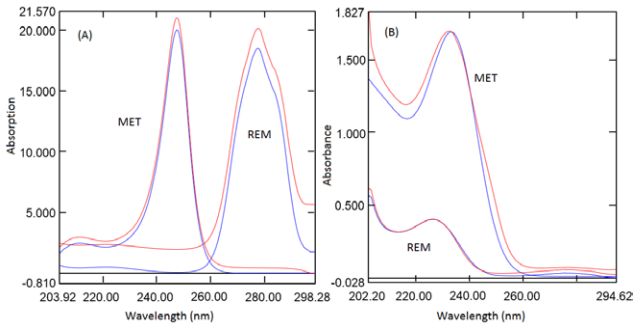
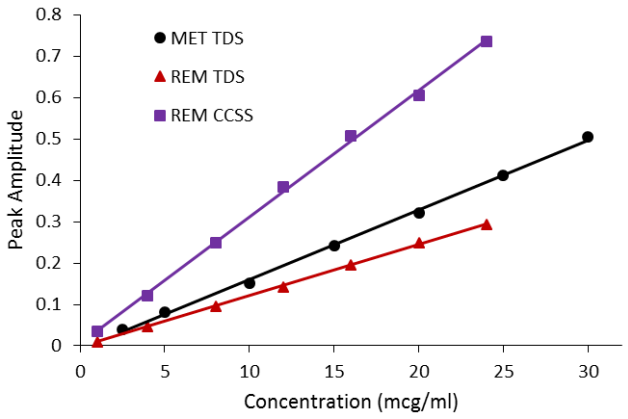
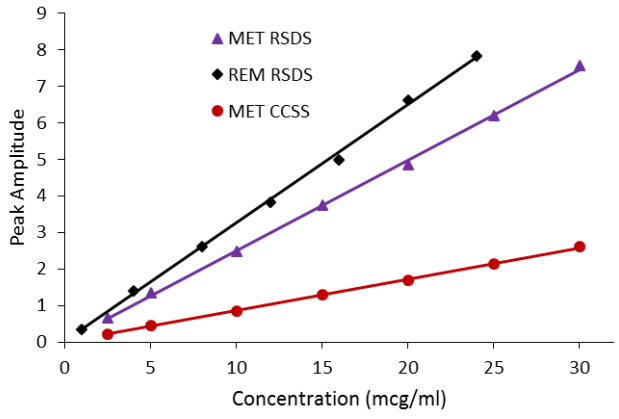


Figure 7: Ratio spectra of MET and REM of pure and mixture (A); zero-order spectra of pure (Blue) and mixture (Red) of MET and REM generated after multiplication showing same absorbance (B).





Supplementary File S2: Calibration curves for MET and REM by all three methods.

generated and the corresponding regression equations were generated. (Table 1).

Limit of detection and limit of quantification were computed using the corresponding equations 3.3 d/s and 10 d/s. Where *d* is the standard deviation of the response and *s* is the slope of the calibration curve. The calculated LOD and LOQ values are tabulated in Table 1.

Precision

The precision of the methods was determined as inter day and intraday precision by investigating both analytes at three different concentration (low, medium and high) to cover the entire linearity range. Intraday precision was performed by analyzing three concentrations of REM and MET, in triplicate on the first day, further, for the inter-day these solutions were assessed for three successive days by all three methods. The precision was presented as percent relative standard deviation and presented in Table 2. The low % RSD found by all three methods indicated the precision of the developed techniques.

Accuracy and specificity

Table 1: Calibration curve results for REM and MET for the proposed methods.

Parameters	Third Derivative Absorbance method		Ratio Second Derivative method		Constant Center Subtraction method	
Drugs	**REM**	**MET**	**REM**	**MET**	**REM**	**MET**
Wave length [nm]	234.8	240.1	277.2	246.6	226.2	232.9
Linearity Range [µg/ml]	1 - 24	2.5 - 30	1 - 24	2.5 - 30	1 - 24	2.5 - 30
LOD [µg/ml]	0.31	0.76	0.23	0.69	0.28	0.81
LOQ [µg/ml]	0.94	2.18	0.74	1.96	0.83	2.42
Slop [m]	0.0124	0.0168	0.3232	0.2471	0.0305	0.0856
Intercept [c]	-0.0031	-0.0076	0.0252	0.0407	0.0066	0.0076
Correlation Coefficient [r^2]	0.9993	0.9985	0.9983	0.9992	0.9987	0.9994

Table 2: Precision results for REM and MET by proposed methods.

		Inter-day			Intra-day		
	Amount of Drug [µg /mL]	**Amount found Mean [*n*=3] ± SD**	**%RSD**	**%RE**	**Amount found Mean [*n*=9] ± SD**	**%RSD**	**%RE**
Third Derivative Absorbance method							
REM	1.00	0.98±0.01	1.02	-2.00	1.01±0.01	0.99	1.00
	12.00	11.89±0.12	1.01	-0.92	12.06±0.08	0.66	0.50
	24.00	23.87±0.29	1.21	-0.54	23.81±0.15	0.63	-0.79
MET	2.50	2.52±0.04	1.59	0.80	2.49±0.04	1.61	-0.40
	15.00	14.81±0.09	0.61	-1.27	14.77±0.18	1.22	-1.53
	30.00	29.74±0.42	1.41	-0.87	30.15±0.32	1.06	0.50
Ratio Second Derivative Spectroscopic method							
REM	1.00	1.01±0.02	1.98	1.00	0.99±0.01	1.01	-1.00
	12.00	11.79±0.16	1.36	-1.75	11.81±0.09	0.76	-1.58
	24.00	23.68±0.22	0.93	-1.33	23.78±0.22	0.93	-0.92
MET	2.50	2.46±0.03	1.22	-1.60	2.51±0.02	0.80	0.40
	15.00	14.88±0.17	1.14	-0.80	14.73±0.19	1.29	-1.80
	30.00	29.79±0.38	1.28	-0.70	29.57±0.42	1.42	-1.43
Constant Center Spectrum Substation method							
REM	1.00	0.99±0.01	1.01	-1.00	1.01±0.01	0.99	1.00
	12.00	12.06±0.11	0.91	0.50	11.81±0.13	1.10	-1.58
	24.00	23.85±0.25	1.05	-0.62	23.66±0.29	1.23	-1.42
MET	2.50	2.46±0.01	0.41	-1.60	2.49±0.04	1.61	-0.40
	15.00	14.88±0.2	1.34	-0.80	14.83±0.23	1.55	-1.13
	30.00	29.55±0.41	1.39	-1.50	29.63±0.34	1.15	-1.23

Accuracy of the projected procedures was assessed by the standard addition method and represented as percent recovery and percent relative error. To determine the percent recovery a known amount of standard solutions of REM and MET at three levels (50%, 100% and 150%) were transferred to an earlier evaluated sample. These solutions were investigated by proposed methods by following the above-described procedures and the concentration of each analyte by each method was calculated by using corresponding regression equations. The experiments were performed in triplicate and the mean percentage recovery (Table 3) was found to be from 99.08% to 100.15% for MET and from 98.73% to 100.27% for REM by all three methods. The low % RE (Table 2) also showed the acceptable accuracy of the proposed techniques. Further, the good percentage recovery of added analytes to the formulation showed no interference from the tablet excipients, indicated the specificity of the methods.

Table 3: Recovery study results.

Drug	Amount added[a] [µg/ml]	% Recovery		
		TDA	RSDS	CCSS
REM	1	100.89	98.57	101.25
	2	98.97	99.15	100.44
	3	100.28	98.46	99.12
	Across Mean	100.05	98.73	100.27
	%RSD	0.98	0.37	1.08
MET	5	98.82	99.79	100.67
	10	100.24	101.58	98.29
	15	98.19	99.08	99.48
	Across Mean	99.08	100.15	99.48
	%RSD	1.05	1.29	1.19

[a]an amount added to the previously analyzed sample (REM 2 µg/ml and MET 10 µg/ml)

Stability studies

Stability studies were performed in terms of benchtop for 24 hr at room temperature and refrigerated sample for 7 days. No change in the assay results was observed even after 24 hr (short term) and 7 days (long term) indicating the stability of analytes in the experimental and refrigerated conditions respectively.

Application of proposed methods to formulations

The recommended UV derivative spectroscopic techniques were applied for the concurrent determination of REM and MET from the solid dosage form (Table 4). The mean percentage recovery was found to be from 99.77% to 101.83% for REM and 98.41 % to 99.85 % for MET and it is in agreement with the amount of analytes in the formulations. Further, the suggested techniques were statistically compared to the reported HPLC method[26] concerning accuracy and precision. No significant discrepancy was witnessed in the assay outcomes acquired by the suggested UV derivative techniques and reported HPLC method. The students' *t*-test and F values were also lower than the critical values.

CONCLUSION

Three simple, accurate and reproducible UV derivative analytical techniques were established for the concurrent quantification of REM and MET from the pharmaceutical preparations. Proposed methods are ecofriendly and economical because dilute-ethanol was used as a solvent, no sophisticated instrument was required and software supplied with the UV

Table 4: Formulation Assay results and statistical results of REM and MET by proposed and reference methods.

	Remogliflozin (%recovery)				Metformin (%recovery)			
	Ref method[c]	TDA	RSDS	CCSS	Ref method[c]	TDA	RSDS	CCSS
REM:MET 100:1000	100.67	99.26	101.05	98.78	100.24	98.65	99.06	98.55
REM:MET 100:500	99.19	98.20	98.93	100.32	98.48	99.72	100.83	99.07
Mean[a]	100.04	99.77	99.94	101.83	100.00	99.12	98.41	99.85
S.D	1.28	1.07	1.09	1.14	0.80	0.98	1.12	1.07
N	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Variance	1.64	1.14	1.18	1.30	1.42	0.95	1.27	1.14
Student's t test (2.22)[b]		0.39	0.13	0.29		1.39	0.88	0.22
F (5.05)[b]		1.43	1.38	1.26		1.49	1.12	1.24

[a] mean of six determinations; [b] critical vales at p=0.05; SD : standard deviation; [c] Reference method:[26] Chromolith (50 mm × 4.6 mm i.d. 5 µm) C_{18} column, Mobile phase acetonitrile and mixture of 25 mM sodium dodecyl sulfate, 10 mM potassium dihydrogen phosphate (pH 3.5) in a ratio of 42%:58% (v/v) respectively, 2 ml /min flow rate, at 230 nm.

spectrophotometer has been used for processing of scanned UV spectra. Very simple manipulation techniques such as ratio derivative and third derivative spectroscopic methods were applied for the concurrent determination of REM and MET without the prior separation. Excellent recovery from the formulation with low %RSD and % RE indicated no intervention from the tablet adjuvants. Further, suggested procedures are simple and rapid, hence could be utilized for quality control of MET and REM formulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

REM: Remogliflozin Etabonate; **MET:** Metformin Hydrochloride; **UV:** Ultraviolet; **%RE:** Percentage Relative Error; **%RSD:** Percent Relative Standard Deviation; **ICH:** International Conference on Harmonisation; **HPLC:** High-performance liquid chromatography; **UPLC:** Ultra high performance liquid chromatography; **LOD:** Limit of detection; **LOQ:** Limit of quantification.

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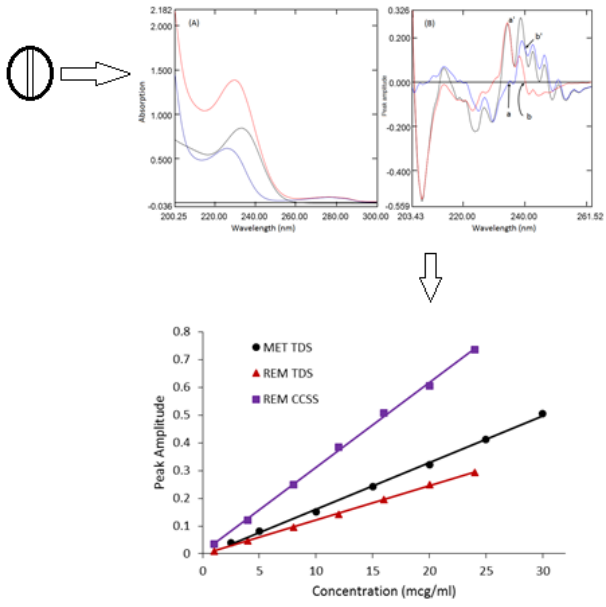
PICTORIAL ABSTRACT



SUMMARY

Three smart, reproducible and non-sophisticated UV derivative spectroscopic procedures were established for the concurrent determination of metformin and remogliflozin by manipulation of UV spectra. Validation of proposed techniques exhibited linearity in the concentration of 2.5 to 30 μg/ml and 1 to 24 μg/ml for MET and REM correspondingly by all three methods. The average percentage of recovery showed was in the range of 99.08% to 100.15% for MET and 98.73% to 100.27% for REM. Further, both analytes were quantified from the formulation using proposed spectroscopic methods with high accuracy. Compression of all three methods with the reported HPLC method showed no variations in the assay results. Hence the suggested techniques could be utilized for regular quality control of formulation consisting of MET and REM.

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