IJBPAS, October, 2022, 11(10): 4793-4811

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MULTIVARIATE CALIBRATION TECHNIQUE AIDED UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ALOGLIPTIN AND REMOGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORM

DEEPIKA REDDY I, SEETHARAMAN R*, KOKILAMBIGAI KS, KAVITHA J AND LAKSHMI K S

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu District, Tamil Nadu - 603203, India

***Corresponding Author: Seetharaman Rathinam: E Mail: seerampharm@gmail.com**

Received 17th Nov. 2021; Revised 24th Dec. 2021; Accepted 10th Feb. 2022; Available online 1st Oct. 2022

https://doi.org/10.31032/IJBPAS/2021/11.10.6502

ABSTRACT

The present work is to create a UV spectrophotometric method for estimating Alogliptin and Remogliflozin etabonate in pharmaceutical tablets using a multivariate calibration technique. The multivariate calibration method determines the absorbance at several wavelengths, enabling more efficient measurement. The ultraviolet spectrophotometric approach was developed and validated. All validation parameters adhere to ICH guidelines. The assay findings obtained with the present method were comparable to those obtained with previously published methods, and no statistical difference was seen. The proposed MVC technique can be used to estimate Alogliptin and Remogliflozin etabonate on a routine basis.

Keywords Alogliptin, Remogliflozin etabonate, Multivariate Calibration, UV Spectrophotometry, Wavelength

INTRODUCTION

In recent years, diabetes has become a major condition that affects people globally. According to the International Diabetic Federation (IDF), there are currently 382 million diabetes patients in the world, with this figure expected to climb to 592 million by 2035. Type-II diabetes is a persistent, progressive condition marked by insulin resistance and B-cell dysfunction (2e4) **[1]**. In this type of diabetes, there are two associated problems: one is an irregularity in insulin hormone

production by the pancreas, and the other is that the cells respond extremely poorly to insulin and the uptake of very little sugar **[2]**. In this study, we chose Alogliptin benzoate and Remogliflozin Etabonate drugs to perform a Multi Variate Calibration (MVC) technique to compare a drug at different Nano meters.

Alogliptin benzoate (ALG) is a new hypoglycaemic medication that belongs to the dipeptidyl peptidase-4-inhibitor (gliptin) family that promotes glucose-dependent insulin release while lowering the risk of heart attack and stroke **[3]**. Chemically ALG is 2-(6-[(3R)-3-aminopyridin-1-yl]-benzonitrile-(3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl-methyl)) benzonitrile; benzoic acid with $C_{25}H_{27}N_5O_4$ as molecular formula and molecular weight as 461.5 g / mol **[4]**. ALG is produces in salt form chemically as benzoate and occurs mostly as the (R)-geometric isomer (greater than 99%). In-vivo, it exhibits minimal or no chiral conversion to the (S)-geometric isomer **[5]**. The commercially available formulations include 6.25 mg, 12.5 mg, and 25 mg tablets for oral use. ALG is an insulin-like hormone that promotes insulin production, inhibits glucagon secretion, delays stomach emptying, increases satiety, and regulates food intake. ALG, a DPP-4 inhibitor, increases glucose-dependent insulin release while decreasing glucagon levels **[6]**. The structure of ALG was represented in **Figure 1**.

Remogliflozin Etabonate (RGE) is an insulin-independent oral hypoglycaemic medication that was recently approved. Chemically, RGE is ethyl [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[5-methyl-1-propan-2-yl-4-[(4-propan-2-yl-oxy-phenyl)-methyl]-pyrazol-3-yl]-oxyoxan-2-yl]-methyl carbonate with $C_{26}H_{38}N_2O_9$ as the molecular formula and the molecular weight as 522.6 g/mol[7]. REM is an inactive prodrug that is transferred into an active form after ingestion and absorption. RGE is not mentioned in any official pharmacopoeia. Non-alcoholic steatohepatitis and type 2 diabetes require this type of medication. The sodium-glucose cotransporter sub-type-2 (SGLT-2) transporter **[8]** is specifically targeted by the RGE drug. This enzyme controls sugar assimilation in the kidneys; hence, obstructing this transporter leads to the excretion of blood glucose through urine **[9]**. Aside from glycaemic control, SGLT-2 antagonists offer a variety of beneficial benefits, including increased body weight, decreased blood pressure, and reduced haemoglobin A1C levels **[10]**. The structure of RGE is represented in **Figure 2**.

The review of literature indicates that various techniques have been published for determining ALG and RGE in

pharmaceutical formulations or biological fluids (single or combined). For ALG, a few chromatographic techniques such as HPLC **[11-13]**, RP-HPLC **[14-21]** spectrophotometric techniques like UV **[22-23]**; hyphenated techniques like LC-MS/MS **[24]**, LC-CAD/LC-UV **[25]**, UPLC-MS/MS **[26]**; conductometric titration **[27]**; and synthesis **[28]** were reported. For RGE, chromatographic techniques like HPTLC **[29]**, RPLC **[30]**, RP-HPLC [31-33]; spectrophotometric techniques like UV **[34]**; hyphenated techniques (RP-HPLC/UV **[35]**, UPLC/PDA **[36]**, RP-UHPLC/DAD **[37]** were reported. No Multi Variate Calibration method was reported for both the drugs. The literature review referred to the MVC technique using UV spectrophotometry, which haven't reported. Hence, the present method deals with the development of the MVC technique for the estimation of ALG and RGE.

The MVC approach was used to decrease instrumental error and increase efficiency. The method is easy, inexpensive and can be applied to bulk chemical and pharmaceutical dosage forms. For exact findings, MVC employs straight regression algorithms ranging between the wavelengths of 5-10nm **[38]**. In this study, we discussed the use of a UV spectral MVC approach with minimal mathematical content for estimating ALG and RGE in pharmaceutical dosage forms. As a result, to ensure the sensitivity in comparison to the traditional ultraviolet (UV) approach, five distinct wavelengths were chosen. The algorithm techniques of MVC's statistics multivariate data are converted into univariate data using the following equations **[39]**:

If the absorbance of a sample(x) is measured at five different wavelengths (λ), that is, at 221, 224, 227, 230, and 233nm, the following equation can be produced for each selected wavelength:

$A_{\lambda 221} = a \times C_x + k_1$ ……. (1)

$A_{\lambda 224} = b \times C_x + k_2$ ……. (2)

$A_{\lambda 227} = c \times C_x + k_3$ ……..... (3)

$A_{\lambda 230} = d \times C_x + k_4$ ……..…. (4)

$A_{\lambda 233} = e \times C_x + k_5$ ……..…. (5)

Whereas,

- A_λ = Absorbance of the sample;
- a, b, c, d, e = Slope of the straight regression functions of a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_x = Concentration of the sample

The above five equations can be rearranged as:

$A_T = a \times C_x + b \times C_x + c \times C_x + d \times C_x + e \times C_x + K_T$……. (6)

Equation (6) can be re- arranged as:

$A_T = C_x (a + b + c + d + e) + K_T$ ……. (7)

Whereas,

- A_T = Sum of the absorbances acquired
- K_T = Sum of intercepts of regression equation

The concentration of the sample (X) in a solution can be calculated by using the equation

$$Cx = \frac{A_T - K_T}{(a+Ab+c+d+e)} \quad \ldots\ldots\ldots\ldots\ldots (8)$$

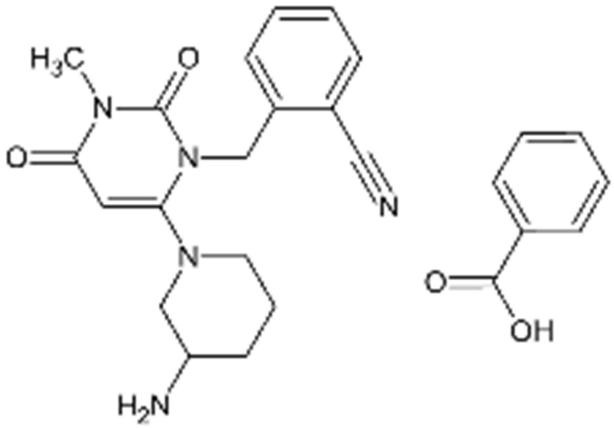


Figure 1: Structure of ALG

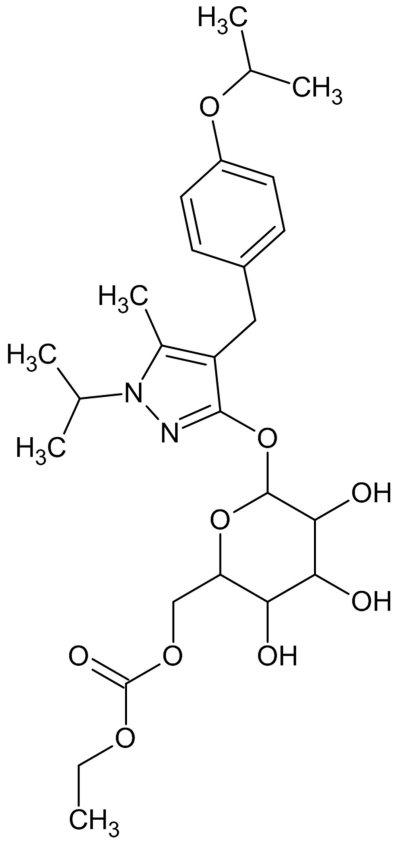


Figure 2: Structure of RGE

MATERIALS AND METHODS

MATERIALS

All the "chemicals and reagents" used in this experiment were analytical grade.

The "active pharmaceutical ingredient" (API) of ALG and RGE with 99.52% and 99.82% purity were received as a gift sample from Ideal Analytical and Research Institution, Puducherry.

Finar Limited (Ahmedabad, India) supplied analytical grade methanol.

Sisco Research Laboratories Pvt. Ltd (Mumbai, India) supplied analytical grade methanol.

A marketed formulation contains each film-coated tablet containing 25mg of ALG from different brands, selected and purchased from a local pharmacy.

A marketed formulation contains each film-coated tablet containing 100 mg of RGE from different brands, selected and purchased in local drug stores.

Instruments used

A LABINDIA UV 3092 model double beam UV-VISIBLE spectrophotometer (Gurugram, India) was used. It consists of an automatic eight-cell charger, Czerny-Turner monochromator optics sealed and coated with quartz, a deuterium lamp, and a tungsten lamp were used as a detector, which has a wavelength of 190-900 nm and a spectral bandwidth of 0.1-5.0 nm with a 0.1nm interval. The software used to run this instrument and produce data output is UV Win Lab Version 5.1.1. For weighing the materials, an analytical balance (AS 245, Mettler Toledo, India) was used, and for sonication purposes, a Soniclean Sonicator (model 106 T, Thebarton, Australia) was used.

Preparation of solutions

Standard stock solution preparation of ALG

Weigh accurately 25 mg of pure ALG API and transfer it into a 25 ml volumetric flask. Dissolve the API in 25 ml of methanol, sonicate for 10 minutes, and then increase the volume with Methanol.

Working solutions of ALG

From the above stock solution, 7-13 µg mL^{-1}solutions were prepared by using Methanol as a solvent.

Standard stock solution of RGE

Weigh accurately 100 mg of pure RGE API and transfer it into a 100 ml volumetric flask. Dissolve the API in 100 ml of methanol, sonicate for 10 minutes, and make up the volume with methanol.

Working solution of RGE

From the above stock solution, 7-13 µg mL^{-1} solutions were prepared by using methanol as a solvent.

Selection of wavelength for MVC

Across the wavelength range of 200 to 400 nm, the ALG working standard solutions were scanned against methanol as the blank solution., which has maximum absorption at 227 nm. Thus, the wavelength for MVC

approach was around these absorption maxima, i.e., 221, 224, 227, 230, 233.

Across the wavelength range of 200 to 400 nm, the RGE working standard solutions were scanned against methanol as the blank solution, which has maximum absorption at 227 nm. Thus, the wavelength for the MVC approach was around these absorption maxima, i.e., 221, 224, 227, 230, 233 nm.

Extraction of drug content from marketed formulation

Preparation of sample solution of ALG

Consider 10 ALG tablets. Weigh each tablet and note the readings. Place those tablets in the motor, and using a pestle, crush them. Weigh the powder, which is equivalent to one tablet of 25 mg, place it in a 25 ml volumetric flask, and add 25 ml of methanol. Sonicate for 10 minutes, making up the volume to the mark with methanol. The solution was filtered through Whatman grade 42 circular filter paper, and appropriate dilutions were made with methanol to achieve concentrations of 7-13 µg mL^{-1}.

Preparation of sample solution of RGE

Consider 10 RGE tablets. Weigh each tablet and note the readings. Place those tablets in the motor, and using a pestle, crush them. Weigh the powder, which is equivalent to one tablet of 100 mg, place it in a 100-ml volumetric flask, and 100ml of methanol is added. Sonicate for 10 minutes, making up the volume to the mark with methanol. Through Whatman grade 42 circular filter papers, the solution was filtered and appropriate dilutions were made with methanol to achieve concentrations of 7-13 µg mL^{-1}.

Stability of the solution

Solution stability studies were performed for ALG and RGE by storing prepared sample solutions at room temperature for 0-12 hours. The absorbance was measured at regular intervals of 0, 6, and 12 hrs.

Linearity

Linearity for ALG and RGE was performed by suitable dilution of the stock solution with methanol to achieve concentrations ranging from 7-13 µg mL^{-1} (0.7, 0.8, 0.9, 1.0, 1.1, 1.2 and 1.3) in order to analyse linearity and spectral region. The absorbance of linearity solutions at the appropriate wavelength was measured and analysed for the MVC method.

Limit of Detection and Limit of Quantification

The Limit of Detection and Limit of Quantification were estimated for ALG and RGE based on the calibration curve slope and standard deviation of responses for a particular wavelength using the following formulae.

$$\text{LOD} = \frac{3.3 \text{ x standard deviation}}{\text{Slop}} \quad \text{…….. (9)}$$

$$\text{LOQ} = \frac{10 \text{ x standard deviation}}{\text{Slop}} \quad \text{…… (10)}$$

Precision

The precision was evaluated and analysed for repeatability through intraday and

interday precision. To test different levels of accuracy, a typical standard solution of ALG and RGE at a concentration of 10 µg ml^{-1} was used. Six solutions at five distinct wavelengths were analysed for the repeatability study. In the scenario of intra variation, at a specified time interval, the absorbance of prepared solutions was evaluated three times on a comparable day. Further, intra variation was accomplished by utilising the absorbance on three subsequent days.

Accuracy

The accuracy of the methodology for ALG and RGE was tested at 80, 100, and 120% of the pre-analysed sample solutions, and the percentages of recovery values were estimated.

RESULTS AND DISCUSSION

The API extraction from the formulation is a challenging task because the API should be extracted completely from the formulation without any loss or interference. These solutions were scanned initially between 210-400 nm and from 300-400 nm there was no absorbance found. The highest spectrum was recorded at 227 nm for both ALG and RGE. To perform MVC, the nm was chosen at 227nm and the UV spectrum was recorded for standards, samples of ALG and RGE by taking methanol as a blank for ALG and for RGE, methanol as a blank. The spectra of 10 µg mL^{-1} standard ALG are represented in **Figure 3**, and for standard RGE in **Figure 4**.

Stability of solution

The results of solution stability of ALG and RGE inferred that is no major changes in the absorbance values as well the spectrum obtained at using the solution measured at 0, 6 and 12 hrs. The absorbance difference between the fresh standard solution and stored solutions were negligible and found to be less than 2%.

Linearity

The developed method linearity findings for ALG were identified a concentration range of 70 - 130% for 10 µg mL^{-1} (7-13 µg mL^{-1}) according to ICH Q2 R1 guidelines. The spectra for ALG were represented in **Figure 6**. The calibration curve was developed by measuring the absorbance of diluted standard solutions at five distinct wavelengths. The calibration graph was plotted for ALG in Figure 5. Within the concentration range the obtained regression equations were calculated, which were found to be linear for ALG with an average of MVC regression equations of $y = 0.0673x + 0.0303$ and $R^2 = 0.999$, which is closer to 1, indicating that the proposed method accepts Beers law. The results are shown in **Table 1**.

The developed method linearity findings for RGE identified a concentration range of 70-130% for 10 µg ml^{-1} (7-13 µg mL^{-1}) according to ICH Q_2 R_1 guidelines. The

spectra for RLG are represented in **Figure 8**. By measuring the absorbance of diluted standard solutions at five distinct wavelengths, the calibration curve was developed. The calibration curve was plotted, which is represented in **Figure 7**. The obtained regression equation was calculated, which was found to be linear for RGE within the concentration range, with an average of MVC regression equations of $y = 0.0668x + 0.0042$ and $R^2 = 0.9998$, which is closer to 1, indicating that the proposed method obeys Beer law. The results are shown in **Table 2**.

Limit of Detection and Limit of Quantification

The Limit of Quantification and Limit of Detection for ALG and RGE were calculated from the linearity slope, which has been confirmed by different sample analyses. The LOD for ALG was calculated from the average of all the absorbance, which was found to be 0.1798 µg ml^{-1} for ALG, and for RGE it was 0.2183 µg mL^{-1}. The LOQ for ALG was calculated from the average of all the absorbance's, which was found to be 0.5448 µg ml^{-1} for ALG, and for RGE it was 0.6616 µg ml^{-1}.

Precision

The system precision spectra for ALG are represented in **Figure 9** and for RGE in **Figure 12**. The interday precision spectra for ALG are represented in **Figure 10**, and for RGE in **Figure 13**. The intraday precision spectra were represented in **Figure 11** for ALG and for RGE in **Figure 14**. The % RSD of system precision, interday and intraday precision, was determined for both ALG and RGE. It was found to be less than 2%, which shows that the approach method is precise. The outcomes are represented in **Table 3** for ALG and in **Table 4** for RGE. The proposed method shows good precision compared to the values obtained from various precision methods.

Accuracy

The accuracy of ALG and RGE was tested at 80, 100, and 120%. The overlay spectra for ALG are in **Figure 15** and for RGE in **Figure 16**. The results are shown in **Table 5** for ALG and in Table 6 for RGE, and the obtained results were found to be within limits.

Assay of marketed formulations:

The quantification of ALG and RGE in the tablet formulation was examined using the suggested spectrophotometric method. For three replicates, the commercial tablet UV absorption spectrum was achieved. The pharmaceutical formulation does not have a significant loss in terms of high analytical recovery values after the extraction and filtration process. The findings, which demonstrate that the new approach performs better than the prior methods, are shown in **Table 7** for ALG and in **Table 8** for RGE. However, the suggested methods

used in other pharmaceutical formulations could be explored. For the proposed and reference methods, the results of ALG were 99.43 ± 0.42 (Mean ± SD) and for RGE, it was 99.42 ± 0.34 (Mean ± SD). For the results to be obtained, the student's T-test for accuracy and the variance ratio F-test for precision were applied. The calculated t-value for ALG is (1.56), as for RGE it is (0.5), which does not exceed the table value. As for ALG, the T-table value was (2.77) and as for RGE, it was (2.77). The F-value for ALG was (1.04) and for RGE was (19), which did not exceed the tabulated values. The F-value for ALG was (19) and for RGE it was (19) at the 95% confidence level for 10 degrees of freedom, which reveals that there was no statistical difference between the reported and proposed methods. The sample spectra of ALG are represented in **Figure 17**, and for RGE in **Figure 18**.

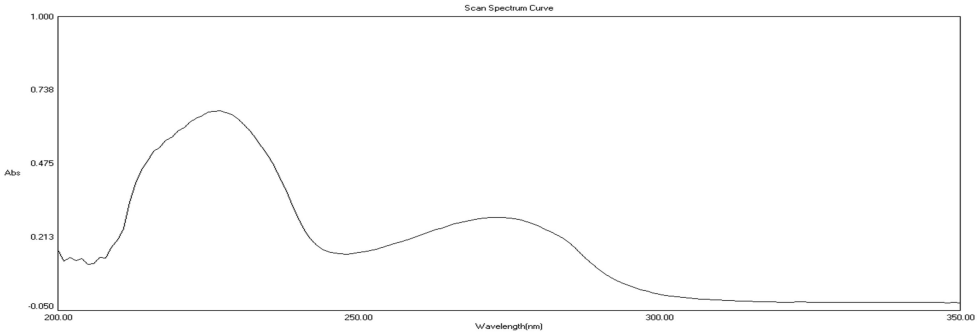


Figure 3: UV spectrum of standard ALG (10 µg mL^{-1}) using methanol as a blank

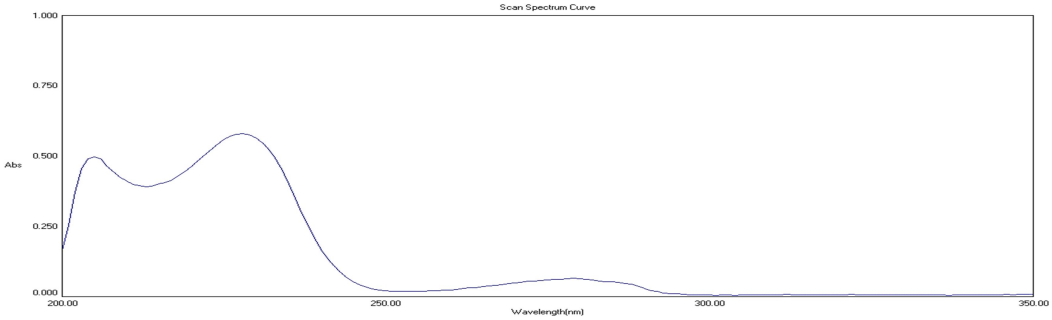
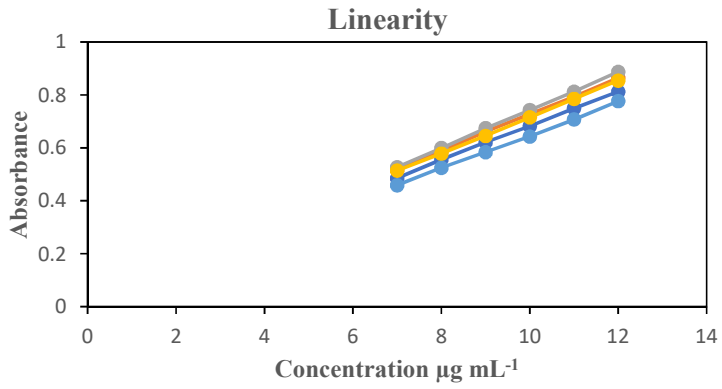
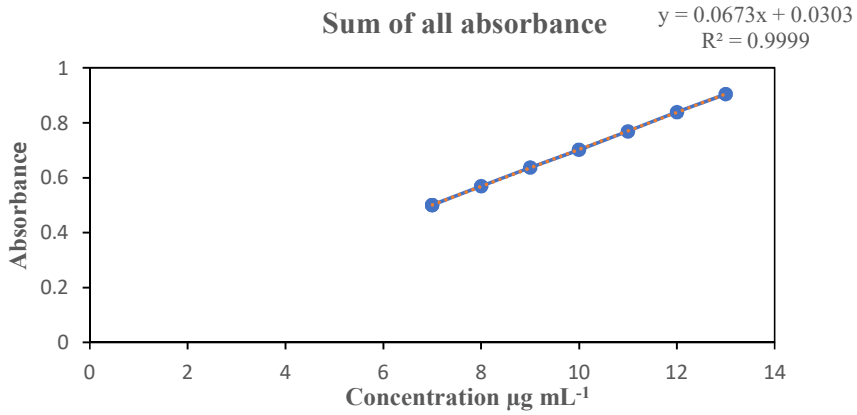


Figure 4: UV spectrum of Standard RGE (10 µg mL^{-1}) by using methanol as blank



(a)



(b)

Figure 5: Multivariate calibration graph (a) and Sum of all absorbance (b) for ALG

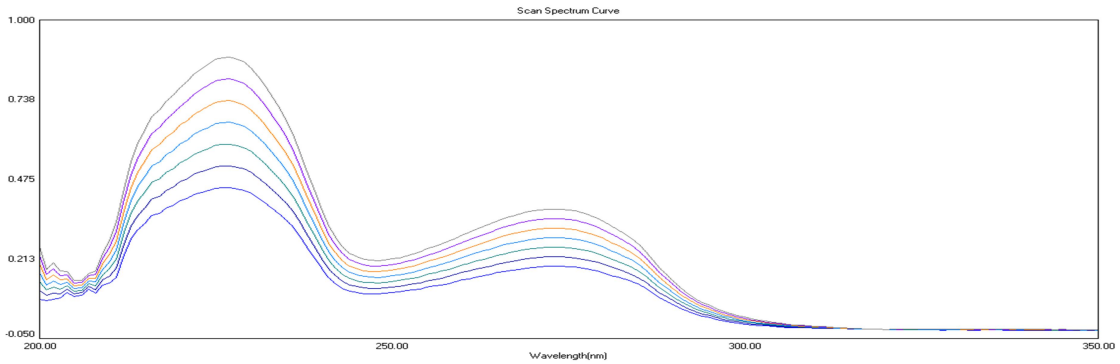
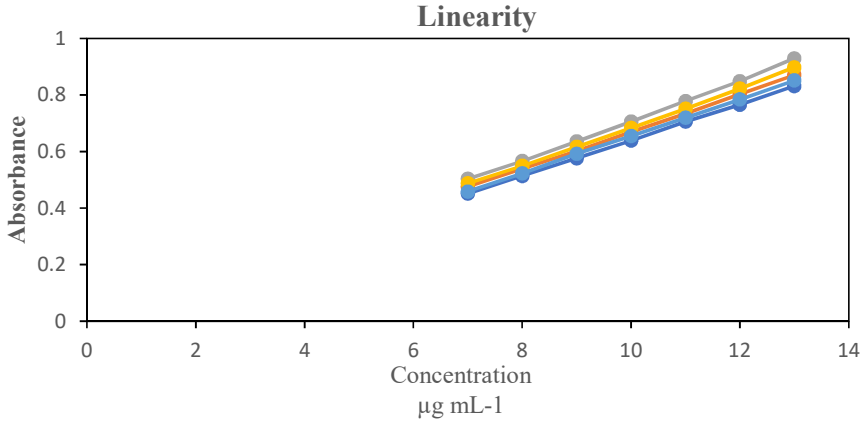


Figure 6: Linearity Spectrum of ALG 7-13 µg mL^{-1} by using methanol as a blank

Table 1: Linearity values for proposed method of ALG

Best-fit values	221 nm	224 nm	227 nm	230 nm	233 nm
Slope	0.0648	0.069	0.0712	0.0685	0.0621
Y-intercept when X=0	0.03457	0.03192	0.02964	0.03092	0.02464
R^2	0.9997	0.9998	0.9998	0.9998	0.9995
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Deviation from zero	Significant	Significant	Significant	Significant	Significant
STEYX	0.0024	0.0023	0.0021	0.0021	0.0033



(a)

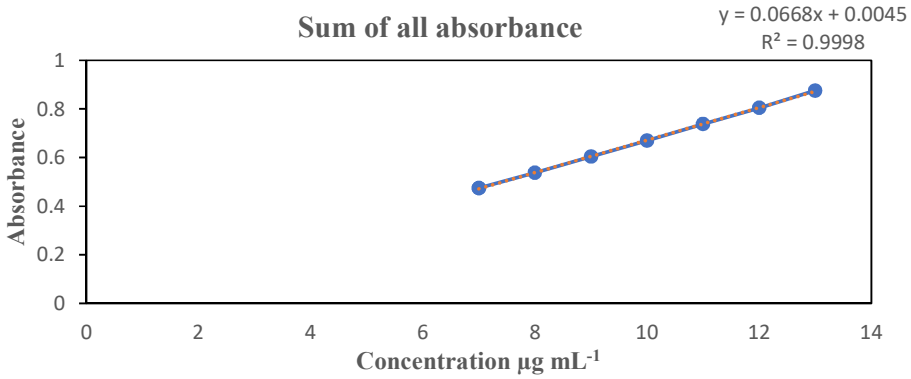


Figure 7: Multivariate calibration graph (a) and Sum of all absorbance (b) for RGE

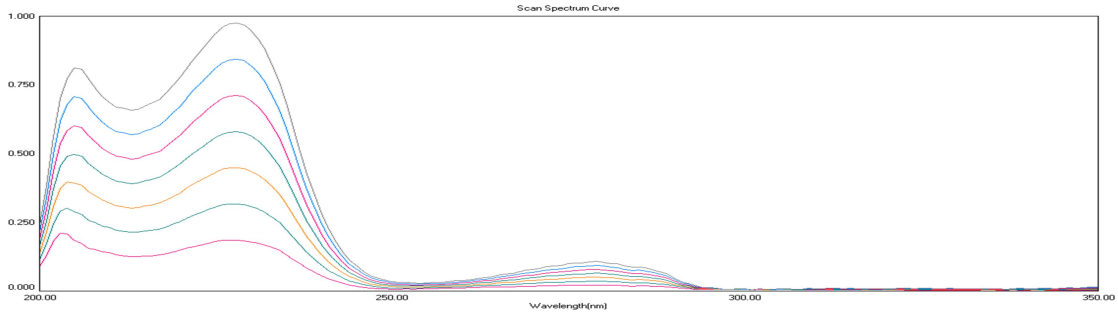


Figure 8: Linearity Spectrum of RGE (7-13 µg mL^{-1}) by using methanol as a blank

Table 2: Linearity values for proposed method of RGE

Best-fit values	221 nm	224 nm	227 nm	230 nm	233 nm
Slope	0.0633	0.0659	0.0708	0.0682	0.0653
Y-intercept when X=0	0.0066	0.0108	0.0002	0.0039	0.0007
r^2	0.9999	0.9998	0.9999	0.9993	0.9999
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Deviation from zero	Significant	Significant	Significant	Significant	Significant
STEYX	0.0015	0.0023	0.0052	0.0043	0.0016

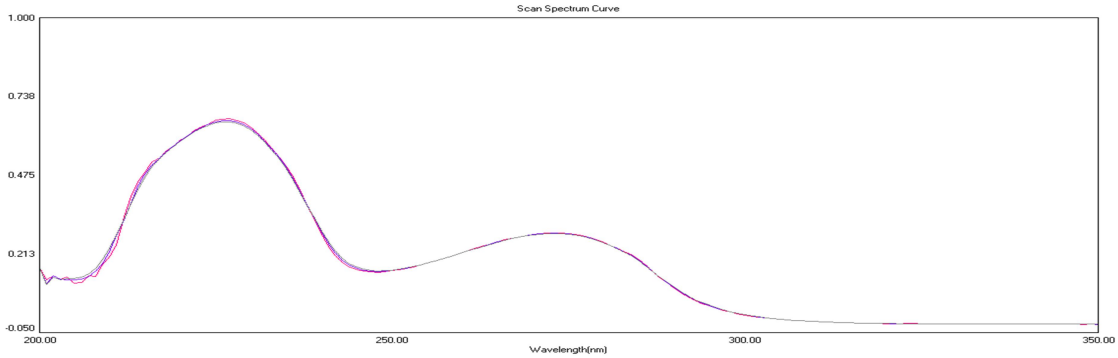


Figure 9: System precision overlay spectra of ALG

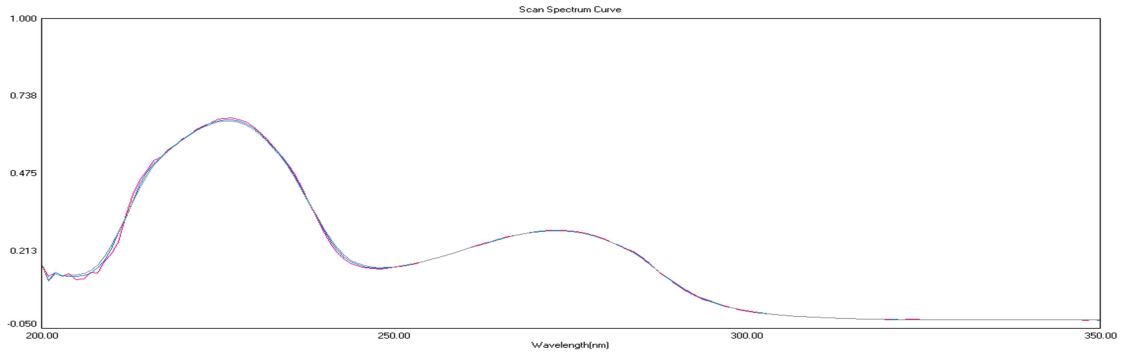


Figure 10: Interday precision overlay spectra of ALG

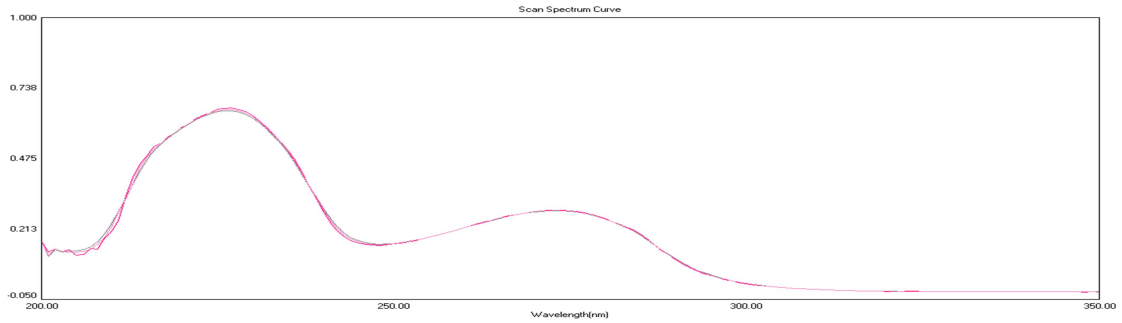


Figure 11: Intraday precision overlay spectra of ALG

Table 3: System precision, Interday and Intraday precision data for the proposed method of ALG

	System precision	Intraday and interday precision		
	Absorbance of standard for 10 µg/ml	% Recovery of sample equivalent to 10 µg/ml of sample		
		Day 1	Day 2	Day 3
1	0.742	98.34	98.55	98.47
2	0.737	99.15	98.82	99.74
3	0.752	99.43	98.42	99.42
4	0.755	98.86	99.78	99.48
5	0.745	99.53	99.27	99.77
6	0.739	98.75	98.51	99.37
Mean	0.745	99.01	99.89	99.38
SD	0.007	0.45	0.53	0.47
%RSD	0.96	0.45	0.53	0.48
Alpha	0.05	0.05	0.05	0.05
n (obser)	6	6	6	6
CI	0.0057	0.3588	0.4268	0.3789

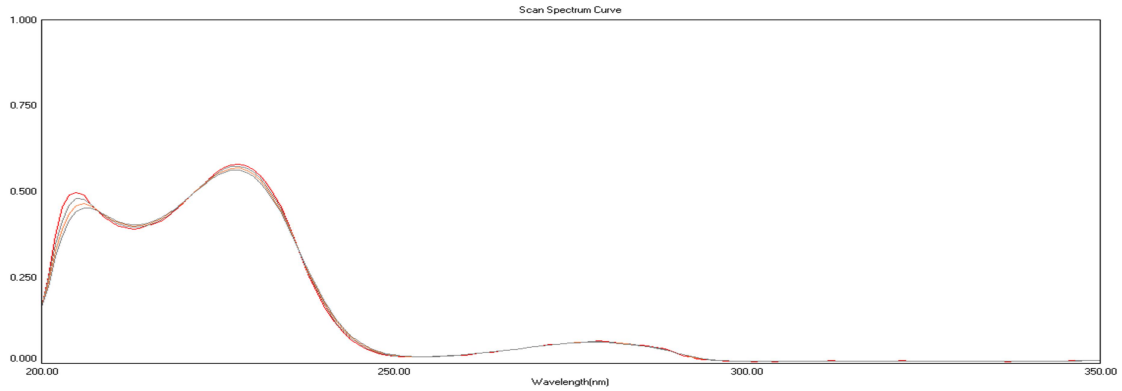


Figure 12: System precision overlay spectra of RGE

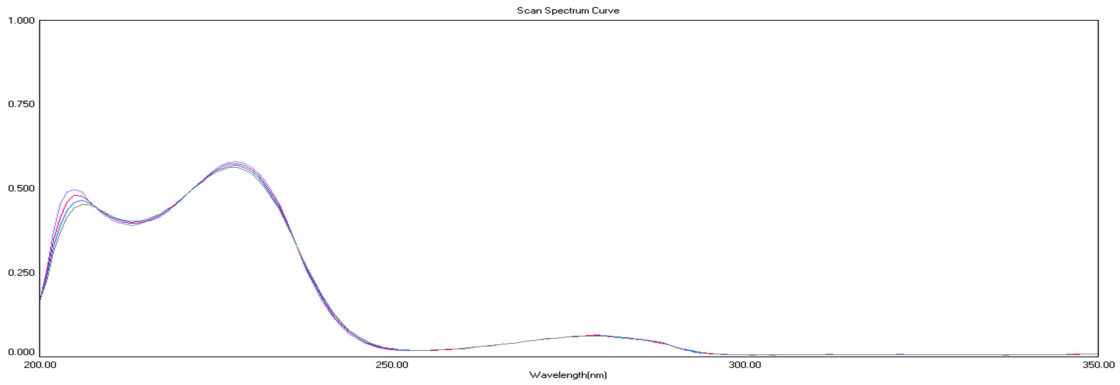


Figure 13: Interday precision overlay spectra of RGE

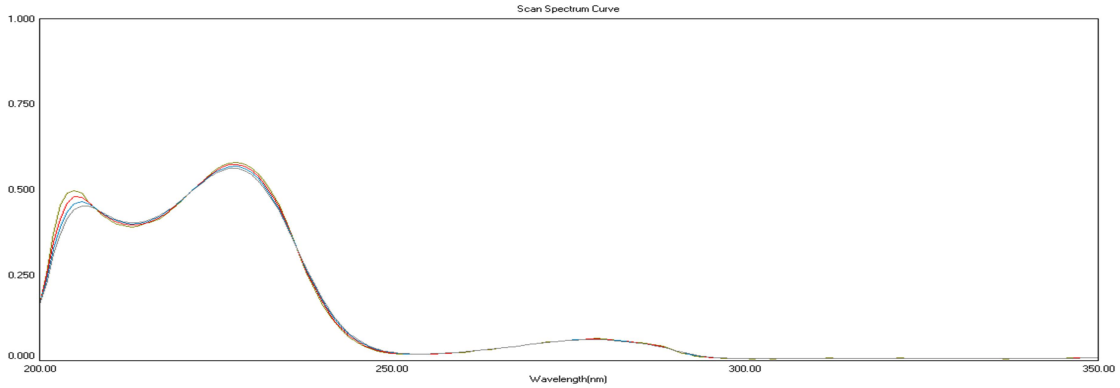


Figure 14: Intraday precision overlay spectra of RGE

Table 4: Interday and intraday precision data for proposed method of RGE

	System precision	Intraday and interday precision		
	Absorbance of Standard 10 µg mL^{-1}	% Recovery of sample equivalent to 10 µg/ml of sample		
		Day 1	Day 2	Day 3
1	0.705	98.94	98.85	98.67
2	0.700	98.58	98.43	99.24
3	0.706	99.42	99.12	98.58
4	0.708	99.76	99.28	99.14
5	0.703	98.83	98.37	99.37
6	0.698	99.65	99.51	99.89
Mean	0.703	99.20	99.89	99.15
SD	0.004	0.48	0.46	0.48
% RSD	0.54	0.48	0.46	0.49

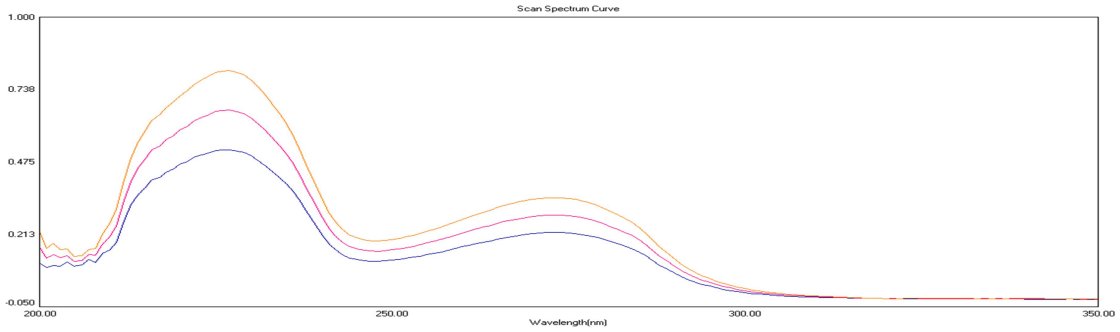


Figure 15: Overlay spectra of accuracy of ALG at 80, 100, 120 % spiking

Table 5: Accuracy data for proposed method of ALG

Concentration levels (%)	Amount present	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Mean % Recovery	SD
80	5	3	7.94	99.29	0.5051
100	5	5	9.83	98.33	1.1015
120	5	7	11.85	98.75	0.7949

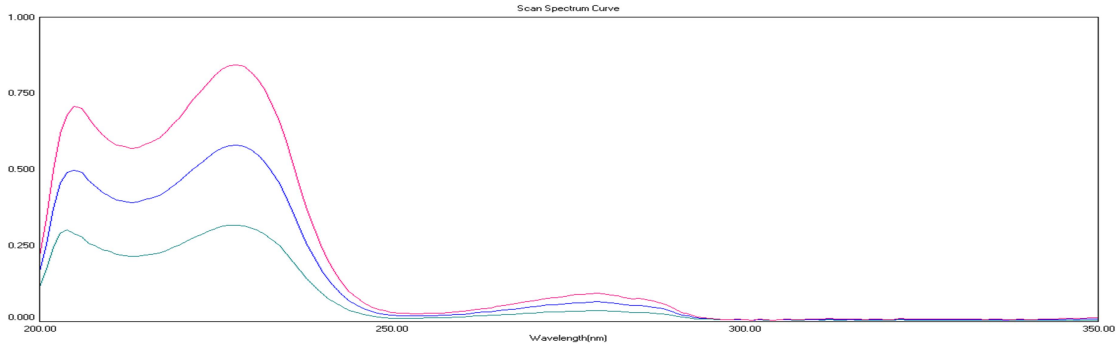


Figure 16: Accuracy overlay spectra of RGE at 80, 100, 120% spiking

Table 6: Accuracy data for proposed method of RGE

Concentration levels (%)	Amount present	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Mean % Recovery	SD
80	5	3	7.90	98.83	0.8322
100	5	5	9.88	98.87	0.3511
120	5	7	11.84	98.69	0.6684

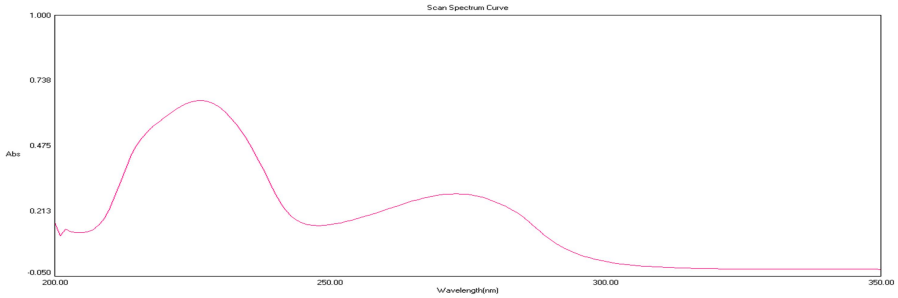


Figure 17: UV spectrum of sample ALG 10 µg mL^{-1} using methanol as a blank

Table 7: Assay results for two marketed formulations of ALG

Marketed formulation	Label claim (mg)	Mean ± SD (n=3)	% RSD
Batch - 1	25	24.86 ±0.08	0.322
Batch - 2	25	24.96 ±0.08	0.327

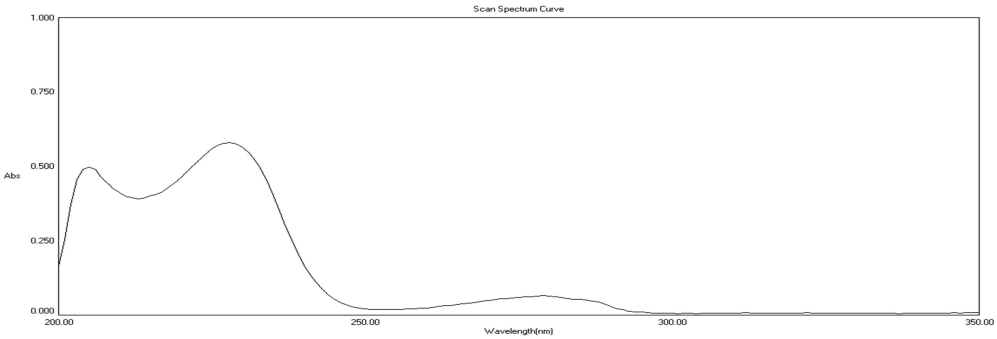


Figure 18: UV spectrum of Sample RGE 10 µg mL^{-1} by using methanol as a blank

Table 8: Assay results for two marketed formulations of RGE

Marketed formulation	Label claim (mg)	Mean ± SD (n=3)	% RSD
Batch - 1	**100**	**99.42 ± 0.34**	**0.340**
Batch - 2	**100**	**98.59 ± 0.34**	**0.386**

CONCLUSION

The proposed MVC approach was a simple, novel, accurate, precise technique for estimating ALG and RGE. It is strongly advised to create a new approach for routine ALG and RGE analysis. All validation parameters were evaluated and confirmed to be within limits when compared to ICH guidelines.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects

ACKNOWLEDGMENTS

The authors are genuinely thankful to management of SRM College of Pharmacy for providing the necessary laboratory facilities to carry out the proposed work

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

FUNDING SOURCES

There is no funding to report

REFERENCES

[1] Kharroubi AT, Darwish HM, Diabetes mellitus: The epidemic of the century, World J Diabetes, 6(6), 2015, 850–67. https://doi.org/10.4239/wjd.v6.i6.850.

[2] Wang C-Y, Neil DL, Home P, An overview of prospects for diabetes management and prevention in the next decade, Diabetes Res Clin Pract. 143, 2018, 101–12. https://doi.org/https://doi.org/10.1016/j.diabres.2018.06.007.

[3] Alogliptin: Uses, Interactions, Mechanism of Action | DrugBank Online n.d. https://go.drugbank.com/drugs/DB06203 (accessed December 1, 2021).

[4] Alogliptin | C18H21N5O2 - PubChem n.d. https://pubchem.ncbi.nlm.nih.gov/compound/Alogliptin (accessed December 1, 2021).

[5] Malathi GMN and S, Alogliptin: A review of Analytical methods, Eur J Biomed Pharm Sci. 8, 2021, 267–71.

[6] Holland DQ, Neumiller JJ, Alogliptin in combination with metformin and pioglitazone for the treatment of type 2 diabetes mellitus, Diabetes, Metab Syndr Obes Targets Ther, 7, 2014, 277–88. https://doi.org/10.2147/DMSO.S37648.

[7] Remogliflozin etabonate | C26H38N2O9 - PubChem n.d. https://pubchem.ncbi.nlm.nih.gov/co

mpound/Remogliflozin-etabonate (accessed December 1, 2021).

[8] Remogliflozin etabonate: Uses, Interactions, Mechanism of Action | DrugBank Online n.d. https://go.drugbank.com/drugs/DB12935 (accessed December 1, 2021).

[9] Mikhail N, Remogliflozin etabonate: a novel SGLT2 inhibitor for treatment of diabetes mellitus, Expert Opin Investig Drugs, 24, 2015, 1381–7. https://doi.org/10.1517/13543784.2015.1061501.

[10] Mohan V, Mithal A, Joshi SR, Aravind SR, Chowdhury S, Remogliflozin Etabonate in the Treatment of Type 2 Diabetes: Design, Development, and Place in Therapy, Drug Des Devel Ther, 14, 2020, 2487–501. https://doi.org/10.2147/DDDT.S221093.

[11] Zhang K, Ma P, Jing W, Zhang X, A developed HPLC method for the determination ofAlogliptin Benzoate and its potential impurities in bulk drug and tablets, Asian J Pharm Sci, 10, 2015, 152–8. https://doi.org/10.1016/j.ajps.2015.01.001.

[12] Naseef H, Moqadi R, Qurt M, Development and validation of an HPLC method for determination of antidiabetic drug alogliptin benzoate in bulk and tablets, J Anal Methods Chem, 2018, 1–7. https://doi.org/10.1155/2018/1902510.

[13] Hammad SF, Abdallah IA, Bedair A, Mansour FR, Salting-out induced liquid–liquid microextraction for alogliptin benzoate determination in human plasma by HPLC/UV, BMC Chem, 15, 2021, 1–20. https://doi.org/10.1186/s13065-020-00729-8.

[14] Madhukar A, Fathima A, Usha A, Satish A, Chary K, Prashanth Kumar A, et al., RP-HPLC Method Development and Validation of Alogliptin Bulk and Tablet Dosage Form, Indo Am J Pharm Sci, 05, 2018, 2897–904.

[15] Yadav Priyanka J, Jadhav Sayali S MSK, Development and validation of RP-HPLC method for Alogliptin Benzoate in bulk drug and dosage form, Int J Pharm Pharm Res, 1, 2014, 1–9.

[16] Vinyas M, Velivela S, Yadav G, Pati NB, Gupta VRM, Analytical method development and validation of alogliptin by RP-HPLC method, Res J Pharm Technol, 9, 2016, 775–8. https://doi.org/10.5958/0974-360X.2016.00148.7.

[17] Runja C, Ravikumar P, Avanapu SR,

Stability indicating RP-HPLC method for simultaneous estimation of alogliptin benzoate and metformin hydrochloride in tablet dosage form, Int J Pharm Pharm Sci. 8, 2016, 116–20.

[18] Daswadkar SC, Roy MA, Walode SG, Mahendra Kumar CB, Optimization of RP-HPLC method for determination of alogliptin benzoate in bulk and dosage form, Int J Chem Sci, 14, 2016, 649–60.

[19] Haribabu B, Rama Krishna Veni P, Bala Murali Krishna K, Lakshmi Prameela K, RP-HPLC Estimation of Alogliptin and Pioglitazone Simultaneously in Combined Tablet Dosage Forms, Marmara Pharm J, 21, 2017, 345–54. https://doi.org/10.12991/marupj.300864.

[20] Snigdha Dhamireddy, DR. Sanjeev Kumar Subudhi VS, RP-HPLC method development and validation of Alogliptin tablet dosage form, Int J Adv Res Med Pharm Sci, 4, 2019, 1–8. https://doi.org/10.52711/0974-4150.2021.00065.

[21] Nemallapudi I, Method development and validation of Simultaneous estimation of alogliptin and metformin hydrochloride by RP-HPLC, Asian J Res Chem Pharm Sci, 6, 2018, 206–14.

[22] Sunil Kumar AVVNK, Reddy T V., Sekharan CB, Spectrophotometric determination of alogliptin in bulk and tablet dosage form using bromate–bromide mixture as brominating agent, Karbala Int J Mod Sci, 3, 2017, 1–10. https://doi.org/10.1016/j.kijoms.2016.12.002.

[23] Chirag, Parle A, Development and validation of UV spectrophotometric method for simultaneous estimation of metformin hydrochloride and alogliptin benzoate in bulk drugs and combined dosage forms, Der Pharma Chem, 6, 2014, 303–11.

[24] G H, SM H, Sensitive LC-MS/MS Method for the Simultaneous Determination of Alogliptin and Voglibose in Human Plasma, J Anal Bioanal Tech, 2017, 08. https://doi.org/10.4172/2155-9872.1000354.

[25] Bertol CD, Friedrich MT, Carlos G, Froehlich PE, Analytical stability-indicating methods for alogliptin in tablets by LC-CAD and LC-UV, J AOAC Int. 100, 2017, 400–5. https://doi.org/10.5740/jaoacint.16-0183.

[26] Rao L, Muralikrishna R, Rao AL, Pharmacy F, Pradesh A, UPLC-MS / MS Assay for Alogliptin in human Plasma and its Pharmacokinetic

Application, Int J Res Pharm Chem, 7, 2017, 558–69.

[27] Bratty MA-, Murayzin H, Almanaa A, Tawhari MQ, Rehman ZU, Alhazmi HA, et al., Quantitative Conductometric Determination of Sitagliptin, Linagliptin, Vildagliptin and Alogliptin by Applying the Concept of Drug-Metal Ion Interaction, Orient J Chem, 35, 2019, 1597–604. https://doi.org/10.13005/ojc/350518.

[28] Yamada M, Hirano S, Tsuruoka R, Takasuga M, Uno K, Yamaguchi K, et al., Development and Scale-Up of an Asymmetric Synthesis Process for Alogliptin, Org Process Res Dev, 25, 2021, 327–36. https://doi.org/10.1021/acs.oprd.0c00544.

[29] Shah DA, Gondalia II, Patel VB, Mahajan A, Chhalotiya U, Nagda DC, Stability indicating thin-layer chromatographic method for estimation of antidiabetic drug Remogliflozin etabonate, Futur J Pharm Sci, 2021, 7. https://doi.org/10.1186/s43094-021-00230-6.

[30] Shah DA, Gondalia II, Patel VB, Mahajan A, Chhalotiya UK, Stability indicating liquid chromatographic method for the estimation of remogliflozin etabonate, J Chem Metrol, 14, 2020, 1–8. https://doi.org/10.25135/jcm.46.20.07.1734.

[31] Mandale DA, Shah C, Jatt R, Development and Validation of Novel RP-HPLC Method for the Simultaneous Determination of Remogliflozin and Vildagliptin in Bulk and in synthetic Mixture, J Pharm Res Int, 33, 2021, 338–49. https://doi.org/10.9734/jpri/2021/v33i40b32296.

[32] K. Likitha Kanna UPP, Stability indicating method Development and Validation of Remogliflozin Etabonate in bulk and pharmaceutical dosage form BY RP-HPLC, Int J Pharm Sci Res, 12, 2021, 4197–207. https://doi.org/10.13040/IJPSR.0975-8232.12(8).4197-07.

[33] Ruchi Vasa, Nimit Vasa, Neha Tiwari, Pragnesh Patani BS, Development and Validation of Stability Indicating Rp- Hplc Method for Estimation of Metformin Hcl and Remogliflozin Etabonate in Pharmaceutical Dosage Form, Int J Pharm Clin Res, 9, 2021, 4079–93.

[34] Attimarad M, Nair AB, Nagaraja S, Aldhubiab BE, Venugopala KN, Pottathil S, Smart UV derivative spectrophotometric methods for simultaneous determination of

metformin and remogliflozin: Development, validation and application to the formulation, Indian J Pharm Educ Res, 55, 2021, S293–302. https://doi.org/10.5530/ijper.55.1s.62 .

[35] Attimarad M, Elgorashe REE, Subramaniam R, Islam MM, Venugopala KN, Nagaraja S, et al., Development and validation of rapid rp-hplc and green second-derivative uv spectroscopic methods for simultaneous quantification of metformin and remogliflozin in formulation using experimental design Separations, 7, 2020, 1–20. https://doi.org/10.3390/separations7040059.

[36] Mohan Rao Tammisetty, Balasekhara Reddy Challa SBP, A Novel Analytical Method for the Simultaneous Estimation of Remogliflozin and Metformin Hydrochloride by UPLC/PDA in Bulk and Formulation Application to the Estimation of Product Traces, Turkish J Pharm Sci, 18, 2021, 296–305.

[37] Patel VA, Pandya C V., Patel ZJ, Patel DR, Pandya AC, Development and Validation of Novel Rp-Uhplc/ Dad Methods for Simultaneous Quantification of Remogliflozin and Metformin in Bulk and Formulation, Rasayan J Chem, 14, 2021, 1384–93. https://doi.org/10.31788/rjc.2021.1426295.

[38] Kannaiah K, Sugumaran A, Eco-Friendly Multivariate Green Analytical Technique for the Estimation of Ketoconazole By Uv Spectroscopy in Bulk and Cream Formulation, Quim Nova XY, 2021, 1–8. https://doi.org/10.21577/0100-4042.20170798.

[39] Kokilambigai, K. S, seetharaman R., Lakshmi KS, Multivariate calibration technique for the spectrophotometric quantification of rasagiline in bulk drug and pharmaceutical formulations, Res J Pharm Technol, 13,2020, 843–9. https://doi.org/10.5958/0974-360X.2020.00159.6.