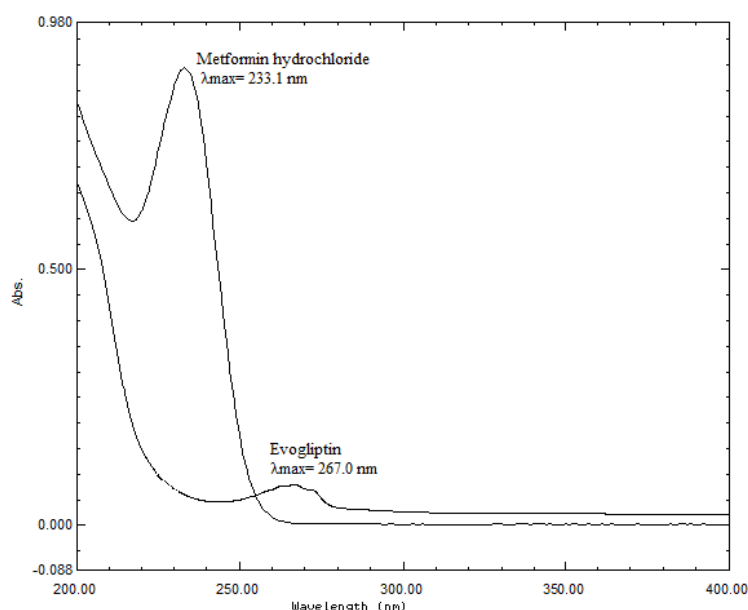


ARTICLE

Eco-friendly UV Spectrophotometric Method for Simultaneous Estimation of Evogliptin and Metformin Hydrochloride in Bulk and Combined Tablet Dosage Form

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Evogliptin (EGT) is used in fixed-dose combination with metformin hydrochloride (MFH) for a better glycemic control in Type 2 diabetes mellitus. To date, no method is available for simultaneous estimation of these drugs. In the present study, an UV spectrophotometric method was developed in distilled water, an environment-friendly solvent using the simultaneous equation technique to simultaneously determine EGT and MFH in bulk and tablet dosage form. The developed method was validated and applied to commercial tablet dosage forms containing EGT and MFH in combination. With a great correlation value ($R^2 > 0.998$), the analytes displayed good linearity in the range of 10–100 $\mu\text{g mL}^{-1}$. The low percent relative standard deviation proved the methods'

precision. The methods' accuracy was demonstrated by excellent recovery. Thus, the developed method was found to be simple, environment-friendly, fast, specific, precise, and accurate, and it may be effectively used for routine analysis of EGT and MFH in bulk and their combined tablet dosage form.

Keywords: evogliptin, metformin hydrochloride, simultaneous equation, UV method, analytical method

Abbreviations used in this paper: EGT: Evogliptin; HPLC: High Performance Liquid Chromatography; HPTLC: High Performance Thin Layer Chromatography; MFH: Metformin hydrochloride; RP-HPLC: Reverse Phase-High Performance Liquid Chromatography; RSD: Relative Standard Deviation; UV: Ultraviolet.

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INTRODUCTION

Metformin hydrochloride (MFH) is chemically N,N-dimethyl imidodicarbonimidic diamide hydrochloride (Figure 1A), a white crystalline hygroscopic powder freely soluble in water.¹ It belongs to the biguanide class and is used in the treatment of Type 2 diabetes mellitus (T2DM). By improving insulin sensitivity, lowering glucose absorption, and blocking hepatic gluconeogenesis, biguanides help lower blood sugar levels. Metformin has the advantage of attaining glycemic control without worsening weight gain or hyperinsulinemia and having a positive effect on blood cholesterol concentrations.²

Patients who do not respond to lifestyle changes and metformin have to add a second oral medication like dipeptidyl peptidase 4 (DPP-4) inhibitors to their treatment plan.^{3,4} DPP-4 inhibitors boost insulin production while suppressing glucagon release by the pancreas' alpha cells by blocking glucagon-like peptide 1 (GLP-1) inactivation.⁵ This helps to bring blood glucose levels back to normal. Because of their easy administration, modest effects on glycosylated hemoglobin (HbA_{1c}), and absence of severe side effects, this class of antidiabetic agents is extensively used either alone or in combination with metformin.⁶ One of the potent and selective DPP-4 inhibitors is Evogliptin which is available as tartrate salt with MFH in fixed dose combination, which significantly improves glycemic control compared to monotherapies.⁷ Evogliptin (EGT) is chemically (3R)-4-[(3R)-3-amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-[(2-methylpropan-2-yl)oxymethyl]piperazin-2-one (Figure 1B) which is freely soluble in distilled water.

The literature survey reveals that many UV, HPLC, and HPTLC methods have been reported for MFH alone⁸⁻¹¹ and in combination with other drugs.¹²⁻¹⁶ For EGT, only one UV and one RP-HPLC method is reported for its estimation.^{17,18} However, no method has been reported for the simultaneous determination of MFH and EGT in combination. UV spectrophotometer methods are favored over other analytical techniques due to their wide range of applications, ease of use, robustness, and simplicity. Furthermore, analysis can be carried out in areas outside of the main laboratory when needed using portable UV spectrophotometers.¹⁹ As a result, the development of such a method might be utilized to estimate the combined dosage form of both medications.

The proposed method is fast, simple, environment-friendly, precise, and reproducible and may be used in tablet dosage for routine analysis of these two drugs simultaneously. The method was developed in distilled water, making the method cheap and environment-friendly. Following International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines, the proposed approach is optimized and validated. There has been a successful attempt in simultaneously estimating both of these drugs utilizing the UV spectrophotometer simultaneous equation approach in this work.

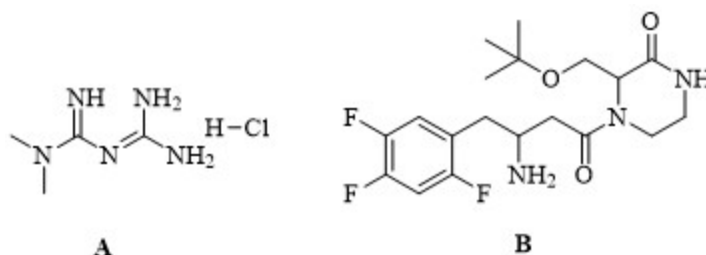


Figure 1. Chemical structure of Metformin hydrochloride (A) and Evogliptin (B).

MATERIALS AND METHODS

Instruments

The experimental study was carried out by a UV/Vis double beam spectrophotometer (UV-1800, Shimadzu) with a 1 cm matched quartz cell and loaded with UV probe software. The study employed a citizen CX 220 weighing balance with 0.1 mg sensitivity for all weighing and a sonicator (Hicon, model 1.5 L (H)).

Reagents and chemicals

The drugs MFH and EGT were procured as gift samples from Anwita Drugs and Chemicals Pvt. Ltd., Telangana and Reine Life Science, Gujarat, respectively. To assess the purity of the drugs, melting point was determined which was found as per the literature. Thus, the drugs were used without further purification. Throughout the study, calibrated glasswares were used. Purified water was acquired using reverse osmosis and filtration via a milli-Q® system (Millipore, Milford, MA, USA) using 0.45 µ membrane and utilized to make all solutions.

Marketed formulation

The marketed formulation studied was Valera M tablets manufactured by Alkem Laboratories Ltd. purchased from the local market. Each tablet contains 500 mg Metformin hydrochloride and Evogliptin Tartrate equivalent to 5 mg Evogliptin.

Preparation of standard stock and working solutions

MFH (10 mg) and EGT (10 mg) accurately weighed were placed into two separate 100 mL volumetric flasks, dissolved in little distilled water, sonicated, and diluted up to 100 mL with distilled water (stock solutions: 100 µg mL⁻¹ MFH and EGT). Then, a working standard solution (10 µg mL⁻¹) of MFH and EGT was prepared by diluting 1 mL of stock solutions to 10 mL with distilled water.

Development of simultaneous equation

To determine the λ_{max}, the working standard solutions of both the drugs were scanned individually across the entire UV range (400–200 nm). Both the drugs displayed considerable absorption at the λ_{max} of another drug; thus, the wavelengths 233.1 nm and 267.0 nm were selected for developing simultaneous equations. Both drugs' stock solutions were diluted separately with distilled water to yield a series of standard solutions ranging from 10 to 100 µg mL⁻¹ for EGT and 1 to 10 µg mL⁻¹ for MFH. The absorbances at the selected wavelengths were measured, and the absorptivities (A 1%, 1 cm) for both drugs at both wavelengths were calculated. The absorbances and absorptivities at these wavelengths can be substituted in equations (1) and (2) to find the concentration of drugs.

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad (1)$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad (2)$$

Where, A₁ and A₂ are the absorbances of sample solutions at 267.0 nm and 233.1 nm, respectively. a_{x1} and a_{x2} are the absorptivities for MFH at 267.0 nm and 233.1 nm, respectively, and a_{y1} and a_{y2} are the absorptivities for EGT at 267.0 nm and 233.1 nm, respectively. C_x and C_y are concentrations of MFH and EGT, respectively in µg mL⁻¹ in the sample solution.

Validation of method

The validation of the analytical procedure was done according to ICH guidelines in terms of linearity, precision, accuracy, ruggedness, and tablet analysis.²⁰

Linearity

From stock solutions of both drugs, ten dilutions were prepared in the range of 10-100 µg mL⁻¹. The absorbances of all the resulting solutions were recorded at the λ_{max} of that particular drug, and a calibration curve was drawn to get the linearity and regression equations.

Precision

The precision of the established method was tested by calculating % relative standard deviation (%RSD) for working standard solutions of EGT and MFH repeated thrice on a similar day (intra-day) and in three days (inter-day). The repeatability test was also done for both the drugs (system Precision), in which a homogenous sample was analyzed six times in the same day.

Accuracy

Three dilutions of the stock mixture solution were prepared to test the accuracy of the developed method. These known concentration solutions were then analyzed as unknown samples using the developed method. To test the developed method's accuracy further, the pre-analyzed sample was separately spiked with an extra 80%, 100%, and 120% of the drug concentrations. The mixtures were analyzed again using the developed method, and %RSD was calculated.

Ruggedness

In this parameter of validation, two analysts performed the same analysis method for the same sample to find out the ruggedness of the method. Then %RSD was calculated.

Analysis of combined tablet dosage form

The developed method was performed on the marketed formulation of EGT and MFH, each tablet containing 5 mg of EGT and 500 mg of MFH. Ten tablets were crushed, extracted with water, and filtered; finally, volume was made up to 100 mL. Further dilutions were made with water to form a working standard solution equivalent to 5 $\mu\text{g mL}^{-1}$ of EGT and 500 $\mu\text{g mL}^{-1}$ of MFH. Aliquots of definite concentration were withdrawn from this working standard solution in six replicates (in Beer-Lambert's Law limit), and absorbance was measured at 267 and 233.1 nm.

RESULTS AND DISCUSSION

Method development

In the current case, the UV spectrophotometric method was established according to the simultaneous equation method. The scanning showed that MFH and EGT exhibit λ_{max} at 233.1 nm and 267.0 nm, respectively (Figure 2). In this study, a partial simultaneous equation method was applied. Absorptivities of both drugs at both wavelengths were calculated by dividing absorbance by concentration ($\mu\text{g mL}^{-1}$) and put in equations 1 and 2. The absorptivity values determined for MFH are 0.004597 (a_{x1}), 0.072336 (a_{x2}) and for EGT, 0.074493 (a_{y1}), 0.004802 (a_{y2}) at 267.0 nm and 233.1 nm, respectively. The equations developed were:

$$C_x = A_1(0.0074493) - A_2(0.004802)/0.005366 \quad (3)$$

$$C_y = A_1(0.072336) - A_2(0.004597)/0.005366 \quad (4)$$

Using the above equations 3 and 4, the concentrations of EGT and MFH can be directly calculated by putting the values A_1 and A_2 (absorbance of the test sample at 233.1 nm and 267.0 nm, respectively) and solving them for C_x (concentration of MFH) and C_y (concentration of EGT).

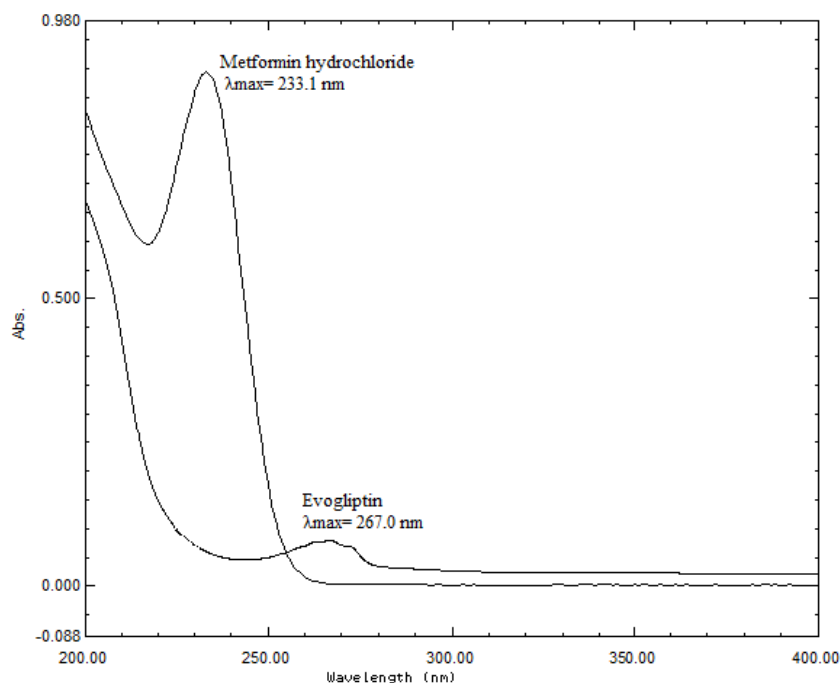


Figure 2. UV Scan of Metformin hydrochloride and Evogliptin.

Method validation

Linearity

The linearity was found to be 10-100 $\mu\text{g mL}^{-1}$ for both EGT and MFH (Table I). The linearity of the established technique was resolute, and the linear regression data for the calibration curve of EGT and MFH showed good linearity ($R^2 = 0.9986$) and ($R^2 = 0.9983$), respectively over the concentration range of 10-100 $\mu\text{g mL}^{-1}$ (Figures 3 and 4).

Table I. Linearity study

Concentration ($\mu\text{g mL}^{-1}$)	Absorbance (Mean \pm SD)	
	Evogliptin	Metformin
10	0.087 \pm 0.000	0.65 \pm 0.03
20	0.151 \pm 0.002	1.34 \pm 0.04
30	0.219 \pm 0.008	2.28 \pm 0.02
40	0.277 \pm 0.002	2.86 \pm 0.03
50	0.367 \pm 0.020	3.85 \pm 0.02
60	0.443 \pm 0.003	4.37 \pm 0.01
70	0.515 \pm 0.003	5.17 \pm 0.01
80	0.579 \pm 0.015	5.94 \pm 0.02
90	0.657 \pm 0.004	6.59 \pm 0.01
100	0.743 \pm 0.008	7.27 \pm 0.04

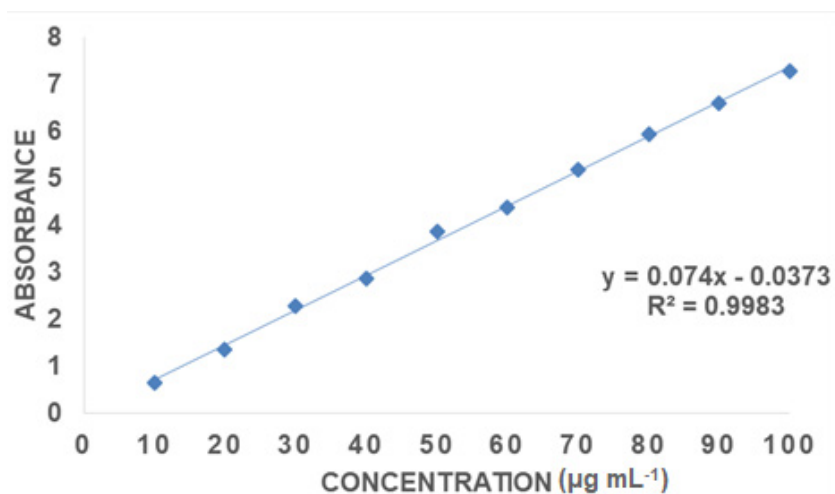


Figure 3. Calibration curve for Metformin HCl.

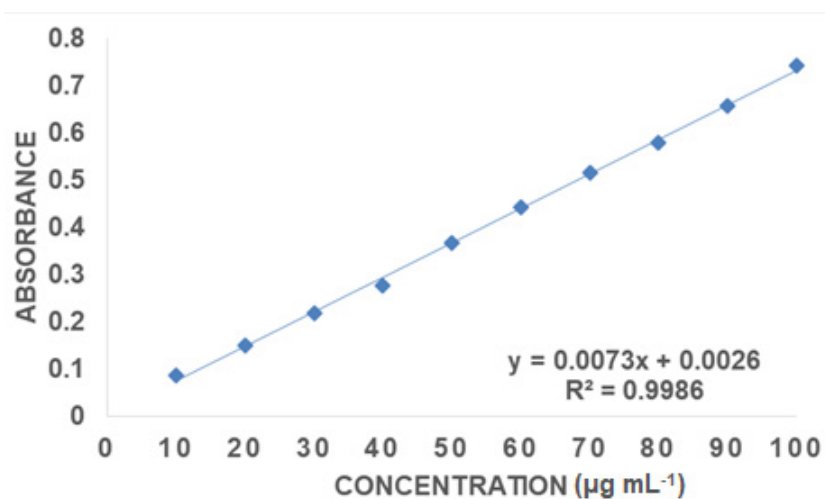


Figure 4. Calibration curve for Evogliptin.

Precision

The developed method was validated for precision by measuring absorbances intra-day and inter-day (Table II) and for repeatability by measuring absorbances six times (Table III).

Table II. Precision Result (Method) for EGT and MFH

	Intra-day study			Inter-day study		
	Morning	Afternoon	Evening	Day 1	Day 2	Day 3
EGT (10 µg mL⁻¹)						
Avg. Abs	0.088	0.086	0.086	0.084	0.082	0.082
SD	0.001	0.001	0.002	0.002	0.002	0.002
%RSD	1.1	1.1	0.9	0.5	0.9	0.6

(continues on the next page)

Table II. Precision Result (Method) for EGT and MFH (continuation)

	Intra-day study			Inter-day study		
	Morning	Afternoon	Evening	Day 1	Day 2	Day 3
MFH (10 µg mL⁻¹)						
Avg. Abs	0.722	0.725	0.0729	0.730	0.723	0.726
SD	0.002	0.003	0.001	0.001	0.002	0.001
%RSD	0.5	0.8	0.5	0.1	0.6	1.2

Table III. Precision Result (System) for EGT and MFH

Concentration (µg mL ⁻¹)	Absorbance	
	EGT	MFH
10	0.087	0.727
10	0.085	0.725
10	0.087	0.723
10	0.083	0.722
10	0.085	0.722
10	0.087	0.725
Mean	0.085	0.724
SD	0.001	0.002
%RSD	1.1	0.2

Accuracy

To test the accuracy and reproducibility, recovery experiments were performed using the standard addition technique by adding 80, 100, and 120% of the drug's concentration in pre-analyzed samples. Data is summarized in Table IV.

Table IV. Accuracy result for EGT and MFH

Level (%)	EGT (10 µg mL ⁻¹)			MFH (10 µg mL ⁻¹)		
	Absorbance	%Recovery	Mean % recovery	Absorbance	%Recovery	Mean % recovery
80	0.065	99.84	99.59	0.592	99.93	99.95
80	0.062	99.90		0.595	99.94	
80	0.061	99.04		0.593	99.98	
100	0.085	100.11	100.31	0.727	100.02	99.87
100	0.087	100.80		0.728	100.03	
100	0.088	100.01		0.725	99.56	

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Table IV. Accuracy result for EGT and MFH (continuation)

Level (%)	EGT (10 µg mL ⁻¹)			MFH (10 µg mL ⁻¹)		
	Absorbance	%Recovery	Mean % recovery	Absorbance	%Recovery	Mean % recovery
120	0.090	100.02	99.93	0.814	100.01	99.94
120	0.091	99.87		0.822	99.98	
120	0.090	99.90		0.815	99.83	

Ruggedness

The developed method was rugged as %RSD was found to be less than 2. The results are reported in Table V.

Table V. Ruggedness result for EGT and MFH

Analyst	EGT (10 µg mL ⁻¹)			MFH (10 µg mL ⁻¹)		
	Absorbance	SD	%RSD	Absorbance	SD	%RSD
Analyst 1	0.087			0.726		
	0.085	0.001	0.1	0.726	0.002	0.2
	0.085			0.722		
Analyst 2	0.084			0.729		
	0.085	0.0005	0.5	0.722	0.003	0.4
	0.085			0.725		

Tablet estimation

The developed method was used to estimate the marketed formulation of the composition of EGT 5 mg and MFH 500 mg. Using the developed simultaneous equations, the concentration of EGT and MFH were estimated in commercial formulation. These data are incorporated in Table VI.

Table VI. Tablet analysis of EGT and MFH using developed method

Formulation	Drugs	Label claim (mg)	Drug found (mg)
Tablets (Valera M 500)	Evogliptin	5	4.96±0.038
	Metformin hydrochloride	500	499.93±0.050

Optical characteristics of UV spectrophotometric method

The summary of optical characteristics and validation parameters of the UV method is shown in Table VII.

Table VII. Summary of optical characteristics of UV method

Parameters	Results	
	EGT	MFH
Detection wavelength (nm)	267.0 nm	233.1 nm
Beer's Law limits ($\mu\text{g mL}^{-1}$)	10-100	10-100
Regression equation	$y = 0.0073x + 0.0026$	$y = 0.074x - 0.0373$
Correlation coefficient	0.9986	0.9983
Slope (m)	0.0073	0.074
Intercept (c)	0.0026	0.0373
Precision (%RSD)		
Intra-day	<2	<2
Inter-day	<2	<2
Accuracy (% mean recovery)		
80% level	99.59	99.95
100% level	100.3	99.87
120% level	99.93	99.94
Ruggedness		
2 Analysts (% RSD)	<2	<2

Discussion

As per literature review, to date, no method is available for simultaneous estimation of EGT and MFH. Thus, the present study focused on developing a simple, cheap, eco-friendly and accurate method for the estimation of these drugs in combination. The UV scanning showed that MFH and EGT exhibit λ_{max} at 233.1 nm and 267.0 nm in water, respectively and both the drugs showed considerable absorbance at the λ_{max} of another drug. Thus, a simultaneous equation was developed by measuring absorbances of EGT and MFH at 233.1 nm and 267.0 nm. The linear regression data for the calibration curve of EGT and MFH showed good linearity ($R^2 = 0.9986$) and ($R^2 = 0.9983$), respectively over the concentration range of 10-100 $\mu\text{g mL}^{-1}$ (Figures 3 and 4). The system and method precision were found within limits according to ICH guidelines. The found values of %RSD were less than 2%, approving the great precision of the established method (Table II and III). The percentage recoveries were close to 100% for this method indicating excellent accuracy of the method (Table IV). For the tablet dosage form, the analysis obtained was in good uniformity in the claimed amount in the marketed sample. Thus, the method developed is simple, accurate, sensitive, and precise and can be used effectively in the simultaneous determination of EGT and MFH in a tablet dosage form.

CONCLUSIONS

In Type 2 Diabetes mellitus, combination therapy gives improved glycemic control when compared to monotherapies. Evogliptin (EGT), a DPP-4 inhibitor, is used in fixed-dose combination with metformin hydrochloride (MFH). However, no method is available for their simultaneous determination. Thus, in the present study, a method was developed using UV spectrophotometry for the simultaneous estimation of

evogliptin and metformin hydrochloride in bulk as well as in tablet dosage form. The method was found to be simple, sensitive, and reproducible. Throughout the estimation, distilled water was used as a solvent, making this method cost-effective and environmentally friendly. The results of the statistical analysis confirmed that the method is accurate and precise. The RSD for all parameters was found to be less than two, establishing the method's validity. Thus, the developed method can be used for routine estimation of evogliptin and metformin hydrochloride simultaneously.

Conflicts of interest

Authors declare no conflicts of interest.

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