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Development and validation of a new spectrophotometric method for simultaneous determination of sitagliptin and metformin hydrochloride in tablet pharmaceutical dosage forms using chemometrics technique in comparison with HPLC

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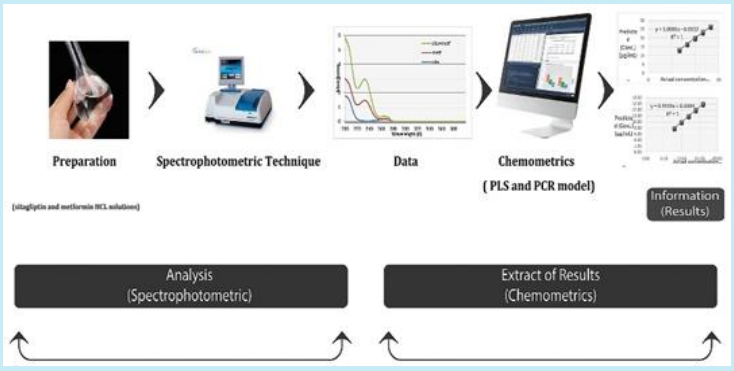
ABSTRACT: A new, quick, easy, affordable and eco-friendly simultaneous spectrophotometric method for determining a combined sitagliptin and metformin hydrochloride in pharmaceutical formulations was developed and validated using two chemometrics technique. These two methods are the partial least square (PLS) and principal component regression (PCR). They do not need to do a sample preparation or separation before analysis. Various drug concentrations and instrumental spectra of 25 mixed solutions of a combination of sitagliptin and metformin hydrochloride were used for model construction in the range of 200–270 nm. The R^2 values of 0.9994 and 0.9996 assigned for the PLS of the sitagliptin and metformin hydrochloride and that of 0.9987 and 0.9996 for the PCR of the sitagliptin and metformin hydrochloride, respectively. It is noteworthy that these two models were successfully and effectively used with the commercial pharmaceutical formulations. Finally, the statistical comparison revealed no significant differences with the results of the HPLC reference method. The proposed method is dependable to be adopted as an alternative analytical method in the pharmaceutical industry's quality control.



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1. Introduction

Chemically, sitagliptin is (3R)-3-purcino-l-[3-(trifiuoromethyl)-5,6-dihydro[1,2,4] triazolo [4,3-a]pyrazin-7 (8H)-yl] -4-(2,4,S-trifiuorophenyl) butan-l-one phosphate monohydrate (Fig. 1a). It is used as dipeptidylpeptidase-4 inhibitor; treatment of diabetes mellitus (British Pharmacopoeia Commission, 2020; Swamy *et al*., 2020).

Metformin HCl is 1,1-dimethylbiguanide hydrochloride and its chemical structure is presented in (Fig. 1b). It is used to treat diabetes mellitus. It is also used to treat polycystic ovarian syndrome. It is taken by mouth and is not linked to weight gain. It is sometimes used as an off-label supplement to help persons who are taking antipsychotics avoid gaining weight (British Pharmacopoeia Commission, 2020).

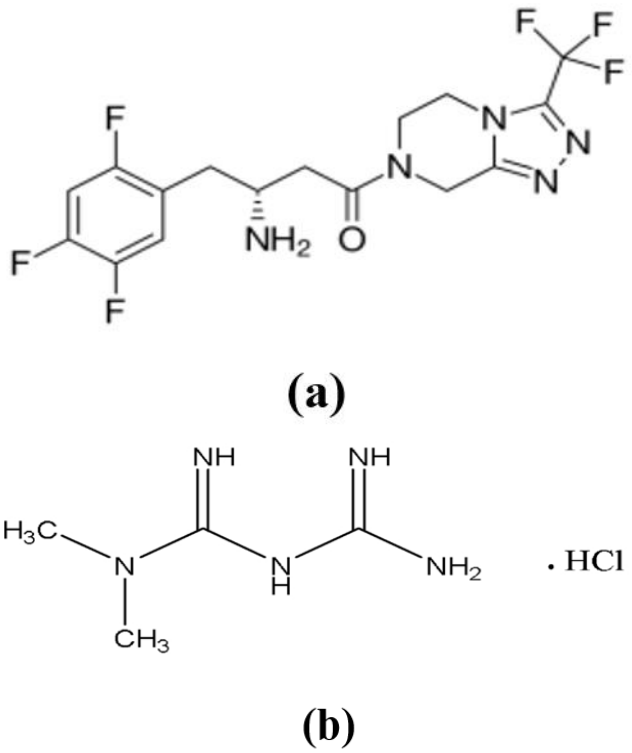

Figure 1. Chemical structure of sitagliptin **(a)** and metformin hydrochloride **(b)**.

Uddin *et al*. (2019) reported that high-performance liquid chromatography (HPLC) is a technique that collects data from simultaneous separation and determination and is more frequently employed in analytical processes for the analysis of pharmaceutical products. However, it has several disadvantages, including the possibility of being bad for the environment and people's health. The HPLC assay also needed a lot of costly chemicals and supplies. Furthermore, it takes a lot of time, which delays the marketing and production operations. The expense of HPLC maintenance is likewise substantial. Spectrophotometry, which is simple, dependable, rapid, economical, and most significantly, environmentally benign, may be a useful option for determining a complicated combination in pharmaceutical quality control laboratories. Additionally, the data show that spectrophotometry and chemometrics in conjugation have a promising future and can be used in place of HPLC in both quantitative and qualitative analysis.

The study of chemometrics has significantly influenced analytical chemistry, notably in the field of spectrum analysis, which is crucial for the quality assurance of pharmaceutical formulations including two or more pharmaceuticals with overlapping spectra (K. Patel *et al*., 2013a; Glavanović *et al*., 2016).

Chemometrics approaches rely on multivariate analysis, which necessitates that ultraviolet (UV) spectrophotometry methods consider multiple variables at once. The absorbance at each wavelength is taken into account, with many wavelengths being taken into consideration (Gandhi *et al*., 2017; R. Patel and Mashru, 2019). The principal component regression (PCR) and partial least squares (PLS) are the two most significant chemometrics techniques utilized in multivariate analysis. For the purpose of determining the combination of medications in pharmaceutical formulations, these multivariate calibration methods employ spectrophotometric data coupled with statistical tools, mathematical models, and software (R. Patel and Mashru, 2019). These techniques additionally rely on the mathematical model's calibration using the absorbance data of calibration standards with known concentrations, which is followed by the prediction of the concentration of unknown samples using those samples' absorbance data (Gandhi *et al*., 2017; R. Patel and Mashru, 2019).

There are many applications for chemometrics in analytical spectroscopy, including UV-visible spectrophotometry (UV-VIS) (Ashour *et al*., 2015; Attia *et al*., 2018; Belal *et al*., 2018; Darbandi *et al*., 2020; Elfatatry *et al*., 2016; Gholse *et al*., 2021; Manouchehri *et al*., 2016; Moussa *et al*., 2021; M. Patel *et al*., 2013b; Phechkrajang *et al*., 2015; Putri *et al*., 2021; Sebaiy *et al*., 2020; 2022; V. D. Singh and V. K. Singh, 2021; Vichare *et al*., 2010), fluorescence spectroscopy (Manouchehri *et al*., 2016; Salem *et al*., 2019; Shinde and Divva, 2015; Walash *et al*., 2011; Zhu *et al*., 2016), NIR spectroscopy (Manouchehri *et al*., 2016; Moroni *et al*., 2022; Muntean *et al*., 2017; 2021; Rahman *et al*., 2020; Sun *et al*., 2021) and FTIR spectroscopic method (Rahman *et al*., 2020). Furthermore, chromatography methods like liquid chromatography (Aminu *et al*., 2019;

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Mohammed *et al*., 2021; Tsvetkova *et al*., 2012; Vu Dang *et al*., 2020) along with a number of other analytical chemistry methods, such as flow-injection analysis are used for the pharmaceutical formulations (Ortega-Barrales *et al*., 2002; Silva *et al*., 2011).

Uddin *et al*. (2019) reported that the majority of the analytes of interest are accompanied in their dosage forms by other compounds that absorb in the same spectral region, making it impossible to distinguish them using the traditional UV spectral studies. It is challenging to use traditional techniques like extraction because they demand a large amount of solvent, which carries risks of analyte loss or contamination as well as the potential for incomplete separation, which is expensive and time-consuming. However, spectrophotometry, as a quick, accurate, low-cost, and easy technology, may be a wonderful choice when used with chemometric techniques for determining a combined mixture in pharmaceutical quality control. When pharmaceutical product quality monitoring calls for dependable, precise, and quick analytical techniques, they are beneficial. This method, which is quick, accurate, and simple to use, avoids the usage of earlier separation procedures.

Many methods for quantifying sitagliptin and metformin hydrochloride have been published, including chromatographic (Adsul *et al*., 2018; Krishnan and Mishra, 2020; Kumar *et al*., 2017) and spectrophotometric approaches (Himabindu *et al*., 2016; Lotfy *et al*., 2015). At the time of writing, we had the following information to our knowledge, there is no reference in the analytical literature reviews for the development and validation of simultaneous spectrophotometric method assisted chemometrics methods for the determination of sitagliptin with metformin HCl in pharmaceutical dosage form. This study aims to develop and validate an adequate and reproducible simultaneous spectrophotometric assay method for the determination of sitagliptin and metformin HCl in tablet pharmaceutical dosage forms using chemometrics technique.

2. Materials and methods

2.1 Materials and Reagents

The reference standard of sitagliptin (as phosphate monohydrate) and metformin HCl were obtained from Global Pharma Company, Sana'a, Yemen. All reagents and chemicals used for the spectrophotometric methods were of analytical grade and HPLC grade were used for the HPLC method. Deionized water (with specific conductance of 0.05 µS cm^{-1}) was produced in-house and used for the preparation of all samples solutions.

2.2 Instrumentation

Double beam UV-VIS (AnalytiK Jena) model (SPECORD 200) at Sana'a University-Faculty of Science was used for the absorbance measurements. The HPLC system was from JASCO with detector (UV-2070 Plus), pump (PU-2089), an auto sampler (AS-2055 Plus) and a column oven (CO-2067 Plus). Electronic balance (AA-160), Denver Instrument. Electronic balance (GH-252), AND. Electronic balance (GR-120), AND. pH meter (3520), Jenway. Centrifuge (Z326 K), Hermle were also used.

2.3 Development and validations procedures

For the aim of developing an accurate, precise and dependable simultaneous spectrophotometric methods assisted with the chemometrics technique, the analytical methods were established and developed to get the intended results for quantifying the targeted components.

2.3.1 Selection of Solvent

Literature reviews were conducted to identify the proper solvents that aid in dissolving the desired active pharmaceutical ingredients without excipients. Through a series of trial-and-error attempts, a suitable solvent was chosen. Other advantages for selecting the appropriate solvent such as available, easy to use, a cheap, environmentally friendly and for the spectrophotometric method implementation were given a full consideration.

2.3.2 Selection of spectral zones analysis

After the phase of choosing the solvent and before the data is preprocessed, the range of 200–400 nm with a 0.2 nm interval was used to record the individual pure and mixed absorbance spectra of the targeted medicinal components. UV spectra of the mixtures analysis were selected among a suitable wavelength range against a solvent blank providing the greatest amount of information about the two components (Shah and Jasani, 2017).

2.3.3 Construction of the training set

As the training set (calibration set), twenty-five different concentrations of the binary mixture of sitagliptin and metformin HCl were prepared to construct

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the model. These mixtures' absorbencies were measured against a blank at intervals of 0.2 nm between 200 and 400 nm.

2.3.4 Construction of chemometric models

The two multivariate calibration models; the PLS and the PCR analysis were established as follows:

- To begin with, binary mixture absorbencies were measured against a blank, and the spectra were saved and extracted into Microsoft Excel in order to develop models;
- Secondly, using absorption data at chosen spectral zones for analysis at intervals of 0.2 nm, the PCR and PLS models were built using the Minitab 17 program;
- Then, the required number of latent variables was obtained using the leave-one-out cross validation method;
- After that, the calibration samples, constants, and coefficients for each wavelength were calculated in order to calculate the predicted concentrations;
- In the end, the predicted concentrations were compared to the actual concentrations in each sample to compute the assay of binary mixture in each sample;
- The root mean square error of cross-validation (RMSECV), which must be as small as possible for a given model, was determined for each method to assess the precision and accuracy of predictions for the models using the following Eq. 1 (Shah and Jasani, 2017):

$$\text{RMSECV} = \sqrt{\frac{\sum(C\text{act} - C\text{pre})^2}{Ic}} \quad (1)$$

where RMSECV = Root mean square error of cross validation; C_{act} = Actual concentration of calibration set; C_{pre} = predicted concentration of calibration set; and I_c = Total number of samples in calibration set.

2.3.5 Validation and construction of the validation set

In order to validate and assess the performance of the suggested and developed spectrophotometric methods assisted chemometric models, these methods were subjected to validation set. Also, the performance criteria of the developed methods including linearity, accuracy, precision (repeatability) and specificity were validated in accordance with the recommendations of International Conference Harmonization and after that determined.

2.4 Developed analytical method procedures for sitagliptin with metformin HCl determination and comparing with reference methods

The performance of the proposed and developed method was determined in accordance with the method validation results. This method was studied and tested for determination of sitagliptin and metformin HCl in marketed pharmaceutical formulations. And they were compared with analysis results of reference method.

2.4.1 Preparation of standard stock solution

Stock solutions of 1670 μg mL^{-1} of sitagliptin and 1000 μg mL^{-1} of metformin hydrochloride were individually prepared in a 100 mL volumetric flask by dissolving 167 mg sitagliptin and 100 mg metformin hydrochloride separately in water.

2.4.2 Preparation of working standard solution

2.4.2.1 Construction of the calibration (training) set

Twenty-five binary mixtures of sitagliptin and metformin hydrochloride were prepared by transferring different aliquots of their standard stock solutions into a series of 50 mL volumetric flasks. The absorbencies of these mixtures were measured between 200 and 400 nm at 0.2 nm intervals against water as a blank.

2.4.2.2 Construction of the validation set

A set of twelve binary mixtures of sitagliptin and metformin hydrochloride was prepared by transferring different volumes into 50 mL volumetric flasks and the procedure under the construction of the training set was repeated.

2.4.2.3 Preparation of spiked samples

Powdered tablets of 25 mg of sitagliptin and 250 mg of metformin hydrochloride were accurately weighed, transferred to a 250 mL volumetric flask and then 200 mL of water was added, the mixture was shaken for 5 min and with frequent shaking the volume completion to 250 mL with the selected solvent was carried out. The solution was then filtered. A 0.5 mL of the filtrate was transferred into 50 mL volumetric flask and calculated amount of sitagliptin and metformin hydrochloride from standard solutions were spiked into sample solution and then diluted with water up to 50 mL. The absorbance was then measured.

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2.4.2.4 Analysis of marketed formulations

The developed method was applied to the measurement of a commercially available samples. It was carried out using the marketed formulation with concentration of 50 mg sitagliptin and 500 mg metformin hydrochloride. The tablets solution prepared in the sample preparation section was diluted with water to prepare solutions with concentration of 10.68 μg mL^{-1} sitagliptin and of 14 μg mL^{-1} metformin hydrochloride. The spectra of the prepared solutions were recorded and then the developed multivariate models PCR and PLS were applied to determine the concentrations of the sitagliptin and metformin HCl.

2.4.2.5 Comparing the suggested method with reference method

Comparison was carried out with the recovery results of the newly developed methods and that of reference method for each of sitagliptin with metformin hydrochloride according to the United States Pharmacopeia (USP, 43). 80 μg mL^{-1} sitagliptin was prepared by dissolving 80 mg sitagliptin in acetonitrile: dilute phosphoric acid (5:95) in a 100 mL volumetric flask as standard stock solution; 5 mL of sitagliptin of the standard stock solution was transferred in acetonitrile: dilute phosphoric acid (5:95) in 50 mL volumetric flask. A test sample was prepared by placing 10 tablets containing 500 mg of sitagliptin to 500 mL volumetric flask; 500 mL of acetonitrile: dilute phosphoric acid (5:95) as solvent was added and the solution was shaken for 1 h then a portion of the solution was centrifuged for 10 min; 2 mL of the supernatant solution was transferred into 25 mL volumetric flask and diluted with solvent. The standard and the test sample of sitagliptin were injected through an HPLC system with a mixture of acetonitrile: monobasic potassium phosphate buffer (pH adjusted to 2 with phosphoric acid) (15:85) as the mobile phase at flow rate of 1 mL min^{-1} through a C8 column (15 cm × 4.6 mm, 5 μm) and column temperature was 30 °C. The UV detection of the sitagliptin was then carried out at 205 nm (United States Pharmacopeia and the National Formulary, 2020).

Metformin hydrochloride was also determined, according to the USP (34), 200 μg mL^{-1} metformin hydrochloride was prepared by dissolving 40 mg metformin hydrochloride in acetonitrile: dilute phosphoric acid (5:95) in a 200 mL volumetric flask as standard stock solution. A test sample was prepared by placing 10 tablets containing 5,000 mg of metformin hydrochloride to 500 mL volumetric flask; 500 mL of acetonitrile: dilute phosphoric acid (5:95) as solvent was added and the solution was shaken for 1 h then a portion of the solution was centrifuged for 10 min; 2 mL of the supernatant solution was transferred into 100 mL volumetric flask and diluted with solvent. The standard and the test sample of metformin hydrochloride were injected through an HPLC system with a mixture of acetonitrile: monobasic potassium phosphate buffer (pH adjusted to 2 with phosphoric acid) (15:85) as the mobile phase at flow rate of 1 mL min^{-1} through a C8 column (15 cm × 4.6 mm, 5 μm) and column temperature was 30 °C. The UV detection of the sitagliptin was then carried out at 205 nm.

3. Results and discussion

3.1 Method development for sitagliptin and metformin HCl determination

3.1.1 Selection of solvent

In order to choose a suitable solvent, solubility was checked in water, methanol, 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} HCl. The drug was found to be soluble in methanol, water, 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} HCl. Therefore, water was selected as diluent that has striking advantages such as easily available, easy to handle, a cheap and environmentally friendly for implementing the spectrophotometric method and Fig. 2 showed the spectra of the sitagliptin and metformin hydrochloride in water.

3.1.2 Selection of spectral zones for analysis

To determine the overlap spectral zones, the absorbance spectra of the pure sitagliptin and metformin hydrochloride samples, and that sample of the mixed sitagliptin with metformin hydrochloride in water were recorded in the range of 200-400 nm with 0.2 nm interval. For the analysis, the UV spectra of the mixtures were selected for a suitable wavelength range (200–270 nm) against water blank. This range provided a great amount of information about the two components as shown in the sitagliptin with metformin hydrochloride spectra (Fig. 2).

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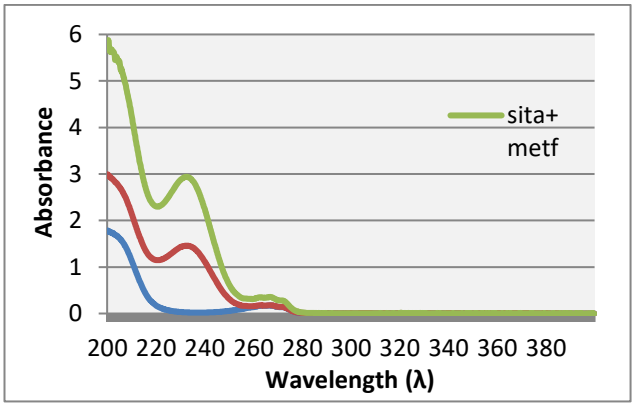

Figure 2. UV absorbance spectra of the pure and mixed samples of sitagliptin and metformin hydrochloride in water solvent.

3.1.3 Construction of the training set

To determine the linear, range from measuring the absorbance at different concentrations for sitagliptin with metformin hydrochloride, the response was found to be linear in the range of 13.36–26.72 μg mL^{-1} for sitagliptin and 8–16 μg mL^{-1} for metformin hydrochloride using 25 different concentrations of sitagliptin and metformin hydrochloride mixtures were prepared to construct the models as shown in Table 1.

Table 1. Composition of calibration set.

Mixture No.	Sitagliptin (μg mL^{-1})	Metformin hydrochloride (μg mL^{-1})	Mixture No.	Sitagliptin (μg mL^{-1})	Metformin hydrochloride (μg mL^{-1})
1	13.36	8	14	20.04	14
2	13.36	10	15	20.04	16
3	13.36	12	16	23.38	8
4	13.36	14	17	23.38	10
5	13.36	16	18	23.38	12
6	16.7	8	19	23.38	14
7	16.7	10	20	23.38	16
8	16.7	12	21	26.72	8
9	16.7	14	22	26.72	10
10	16.7	16	23	26.72	12
11	20.04	8	24	26.72	14
12	20.04	10	25	26.72	16
13	20.04	12			

3.1.4 Construction of chemometrics models

The spectra were saved and extracted into Microsoft Excel for model generation. The PCR and PLS models were developed utilizing the absorption data for the selected spectral zones using Minitab 17 software program. After the PCR and PLS models have been constructed, the optimum number of principal components of sitagliptin and metformin hydrochloride were obtained and given in Tables A1–4 of the Appendix.

3.1.5 Determination of the optimum number of the principal components of sitagliptin and metformin hydrochloride for PLS

Selecting the proper number of principal components for the development of model was necessary to obtain good prediction. Leave-one-out cross validation method was used to obtain the necessary optimum number of the principal factors for the PLS model. It was found that the optimum number of the principal components were eight for sitagliptin and eight for metformin hydrochloride as mentioned above and as given in Table A1 and A2 of the Appendix.

3.1.6 Determination of the constant and coefficients obtained at each wavelength of sitagliptin and metformin hydrochloride for PLS models

The constant and coefficients at each wavelength were calculated using Minitab 17 program as illustrated in Table A1 of the Appendix.

3.1.7 Determination of the predicted concentrations and the recovery of sitagliptin and metformin hydrochloride for PLS models

The predicted or calculated concentrations in μg mL^{-1} of the sitagliptin and metformin hydrochloride were worked out from the multiple regression Eq. 2:

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predicted (Calculated) = Constant + ∑ (Coefficient × Absorbance) (2)

The predicted or calculated concentrations of the components were compared with the actual concentrations and the assay of binary mixture were calculated. RMSECV was calculated and found to be low. The low values of RMSECV in Table 2 indicate both the precision and accuracy of PLS model for sitagliptin and metformin hydrochloride were very high and the R^2 values in Fig. 3 were also of very high linearity.

Table 2. Results of the predicted concentrations with the recovery of sitagliptin and metformin hydrochloride in the binary mixture in each sample for PLS model.

Name	Sitagliptin			Metformin hydrochloride		
Constant	–1.1712			0.4045		
Mixture No.	Actual Conc.	Predicted Conc.	%Recovery	Actual Conc.	Predicted Conc.	%Recovery
1	13.36	13.36	100.00	8.00	7.99	99.88
2	13.36	13.35	99.93	10.00	10.02	100.20
3	13.36	13.36	100.00	12.00	11.98	99.83
4	13.36	13.34	99.85	14.00	14.00	100.00
5	13.36	13.37	100.07	16.00	15.99	99.94
6	16.70	16.73	100.18	8.00	7.99	99.88
7	16.70	16.68	99.88	10.00	10.00	100.00
8	16.70	16.71	100.06	12.00	12.00	100.00
9	16.70	16.67	99.82	14.00	14.01	100.07
10	16.70	16.71	100.06	16.00	16.01	100.06
11	20.04	20.04	100.00	8.00	8.00	100.00
12	20.04	20.04	100.00	10.00	10.02	100.20
13	20.04	20.05	100.05	12.00	12.00	100.00
14	20.04	20.04	100.00	14.00	14.00	100.00
15	20.04	20.08	100.20	16.00	16.00	100.00
16	23.38	23.37	99.96	8.00	8.00	100.00
17	23.38	23.38	100.00	10.00	10.00	100.00
18	23.38	23.37	99.96	12.00	11.99	99.92
19	23.38	23.39	100.04	14.00	14.00	100.00
20	23.38	23.36	99.91	16.00	15.99	99.94
21	26.72	26.71	99.96	8.00	7.99	99.88
22	26.72	26.74	100.07	10.00	10.01	100.10
23	26.72	26.73	100.04	12.00	12.00	100.00
24	26.72	26.71	99.96	14.00	13.99	99.93
25	26.72	26.71	99.96	16.00	16.00	100.00
		Mean%	100		Mean%	99.99
		RSD%	0.09		RSD%	0.089
		RMSECV	0.016		RMSECV	0.01

The linearity of the developed method was tested by constructing a cross-validation of the data in Table 2. The results obtained in Fig. 3 indicated that the developed method possessed high linearity with $R^2 = 1$ within the method linear range (13.36–26.72 μg mL^{-1}) for sitagliptin and $R^2 = 1$ within the method linear range (8–16 μg mL^{-1}) for metformin hydrochloride. The linearity of the developed method was very high and most importantly, environmentally friendly with respect to the solvent (water) used. In comparison, Adsul *et al.* (2018) revealed that the linearity of the HPLC methods which carried out in non-eco-friendly solvents and mobile phases was almost similar to our eco-friendly (water) developed method and better than another HPLC method (Kumar *et al.*, 2017).

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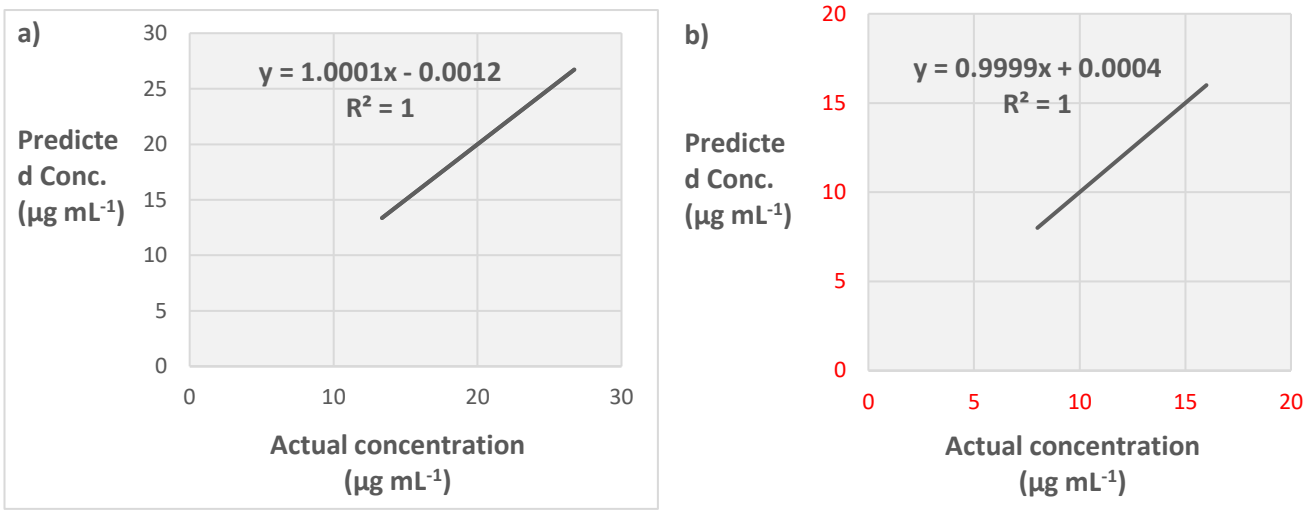

Figure 3. The PLS cross validation for the calibration set of the actual vs. predicted concentration. **(a)** Sitagliptin; **(b)** Metformin HCl.

3.1.8 Determination of the optimum number of the principal components and their coefficients of sitagliptin and metformin HCl for PCR

The PCR was computed by using a few principal components and performed regression analysis of these components with concentration in order to determine the principal components coefficients of sitagliptin and metformin hydrochloride for PCR model as illustrated in Table A4 of the Appendix. From the treatment of the principal component's coefficients in (Table A4 of the Appendix) using Minitab 17 program. Regression equations (Eqs. 3 and 4) of sitagliptin and metformin hydrochloride were obtained and used to calculate the predicted concentration as shown below.

Regression equation of sitagliptin

$$-2.182 + 0.5991\ Z1 + 3.9880\ Z2 + 3.65\ Z3 - 0.92\ Z4 + 1.75\ Z5 - 5.97\ Z6 \quad (3)$$

Regression equation of metformin hydrochloride

$$0.066 + 0.94302\ Z1 - 0.9038\ Z2 - 0.353\ Z3 - 1.524\ Z4 + 1.993\ Z5 + 1.703\ Z6 \quad (4)$$

where Z is the principal components coefficients.

3.1.9 Determination of the predicted concentrations and recovery of sitagliptin and metformin hydrochloride for PCR models

The predicted or calculated concentrations in µg mL^{-1} of the sitagliptin and metformin hydrochloride were calculated from multiple regression Eq. 5:

$$\text{predicted (calculated)} = \text{constant} + \sum(\text{coefficient} \times \text{absorbance}) \quad (5)$$

The predicted or calculated concentrations of the sitagliptin and metformin hydrochloride were compared with the actual concentrations and the assay for binary mixture were calculated in each sample. RMSECV was calculated and found to be low. The RMSECV low values in Table 3 indicate that both the precision and accuracy of PCR model for sitagliptin and metformin hydrochloride were very high, with the R^2 values in Fig. 4 of very high linearity.

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Table 3. Results of the predicted concentrations with recovery of sitagliptin and metformin hydrochloride in binary mixture in each sample for PCR models.

Name	Sitagliptin			Metformin hydrochloride		
Constant	-2.182			0.066		
Mixture NO.	Actual Conc.	Predicted Conc.	%Recovery	Actual Conc.	Predicted Conc.	%Recovery
1	13.36	13.40	100.30	8.00	8.04	100.50
2	13.36	13.15	98.43	10.00	10.07	100.70
3	13.36	13.23	99.03	12.00	11.91	99.25
4	13.36	13.14	98.35	14.00	14.07	100.50
5	13.36	13.39	100.22	16.00	15.98	99.88
6	16.70	16.95	101.50	8.00	7.96	99.50
7	16.70	16.75	100.30	10.00	10.02	100.20
8	16.70	16.61	99.46	12.00	12.02	100.17
9	16.70	16.98	101.68	14.00	13.95	99.64
10	16.70	16.89	101.14	16.00	15.98	99.88
11	20.04	20.05	100.05	8.00	7.96	99.50
12	20.04	20.08	100.20	10.00	9.90	99.00
13	20.04	19.70	98.30	12.00	11.94	99.50
14	20.04	20.01	99.85	14.00	13.96	99.71
15	20.04	20.26	101.10	16.00	16.08	100.50
16	23.38	23.63	101.07	8.00	8.04	100.50
17	23.38	23.45	100.30	10.00	10.10	101.00
18	23.38	23.14	98.97	12.00	12.03	100.25
19	23.38	23.49	100.47	14.00	14.08	100.57
20	23.38	23.50	100.51	16.00	15.96	99.75
21	26.72	26.68	99.85	8.00	8.01	100.13
22	26.72	26.77	100.19	10.00	9.99	99.90
23	26.72	26.55	99.36	12.00	11.95	99.58
24	26.72	26.47	99.06	14.00	13.97	99.79
25	26.72	26.73	100.04	16.00	16.01	100.06
		Mean%	99.99		Mean%	100.00
		RSD%	0.94		RSD %	0.49
		RMSECV	0.169		RMSECV	0.054

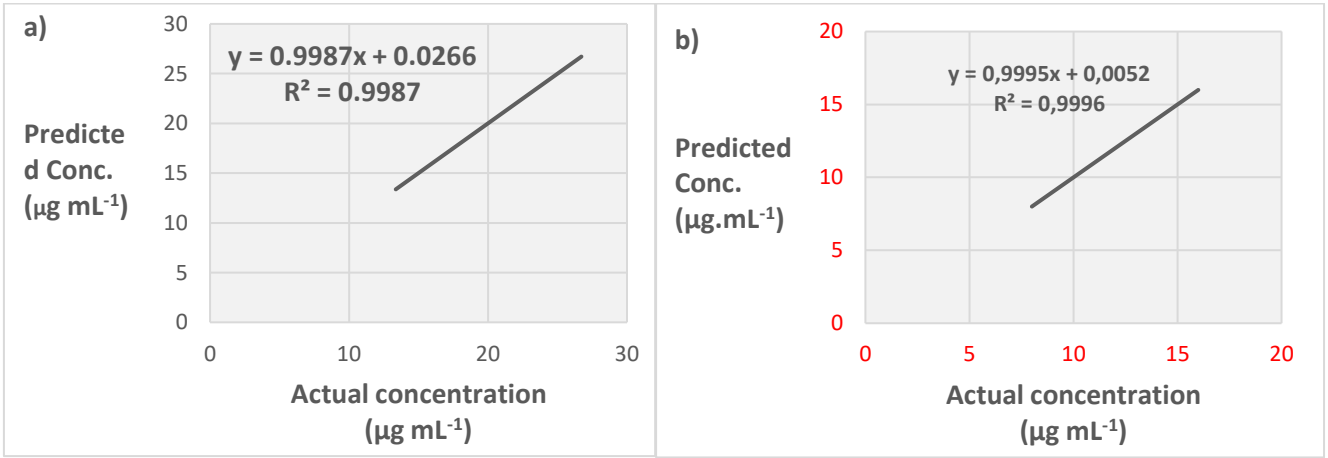

Figure 4. The PCR cross validation for calibration set of the actual vs. predicted concentration. **(a)** Sitagliptin; **(b)** Metformin HCl.

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3.2 Validation method for sitagliptin and metformin hydrochloride

3.2.1 Construction of validation set

The results of prediction and the percentage recoveries are represented in Table 4. The predictive abilities of the models were evaluated by plotting the actual known concentrations against the predicted concentrations that shown in Fig. 5 and 6. A tremendous agreement between the predicted (calculated) and actual concentration of sitagliptin and metformin hydrochloride for PLS and PCR models can be observed in Fig. 5 and 6.

Table 4. Results of validation set of sitagliptin and metformin HCl for PLS and PCR model.

NO.	METHOD		PLS				PCR			
	Sita.	Metf.	Sita.		Metf.		Sita.		Metf.	
	Actual (µg mL^{-1})		Predicted (µg mL^{-1})	%R	Predicted (µg mL^{-1})	%R	Predicted (µg mL^{-1})	%R	Predicted (µg mL^{-1})	%R
1	16.02	8	15.76	98.38	7.71	96.38	16.35	102.06	7.93	99.13
2	16.02	9.6	16.17	100.94	9.36	97.50	16.63	103.81	9.58	99.79
3	13.35	8	13.41	100.45	8.02	100.25	13.55	101.50	8.09	101.13
4	13.35	10	12.96	97.08	9.75	97.50	13.02	97.53	9.85	98.50
5	20.03	10	19.95	99.60	9.80	98.00	20.40	101.85	9.97	99.70
6	20.03	12	20.03	100.00	11.71	97.58	20.56	102.65	11.92	99.33
7	26.7	8	26.66	99.85	7.78	97.25	27.43	102.73	7.98	99.75
8	26.7	10	26.83	100.49	9.58	95.80	27.12	101.57	9.79	97.90
9	16	9.6	16.28	101.75	9.76	101.67	16.33	102.06	9.77	101.77
10	16	12	15.91	99.44	12.03	100.25	15.96	99.75	12.00	100.00
11	24	12	23.91	99.63	11.75	97.92	24.17	100.71	11.92	99.33
12	24	14.4	24.32	101.33	14.09	97.85	24.55	102.29	14.16	98.33
			Mean%	99.91		98.16	Mean%	101.54		99.56
			RSD%	1.22		1.74	RSD%	1.53		1.06

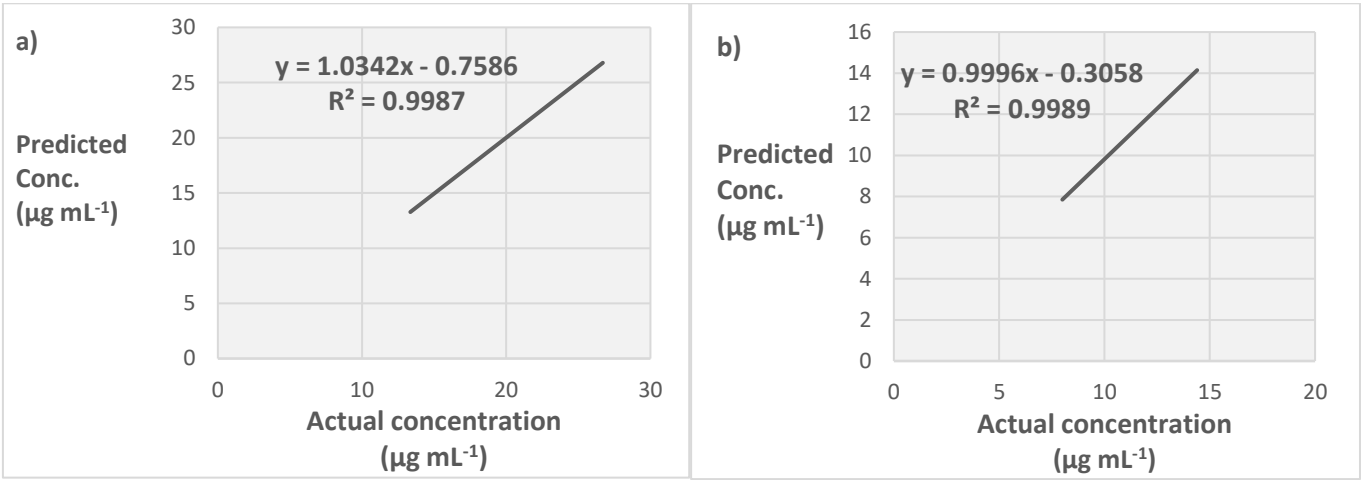

Figure 5. The PLS cross-validation for validation set of the actual vs. predicted concentration. **(a)** Sitagliptin; **(b)** Metformin HCl.

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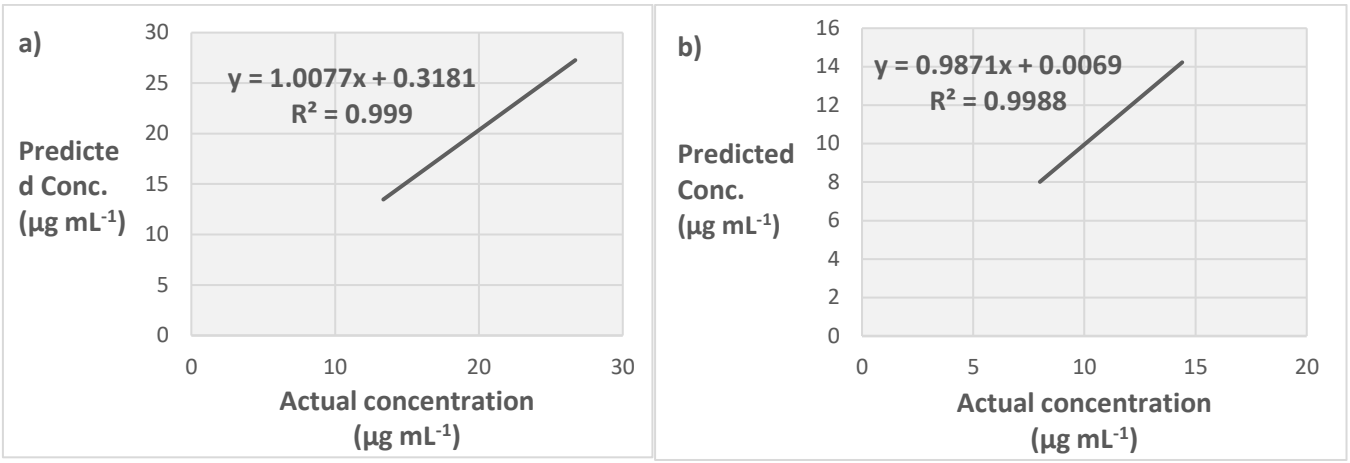

Figure 6. The PCR cross-validation for validation set of the actual vs. predicted concentration. **(a)** Sitagliptin; **(b)** Metformin HCl.

3.2.2 Precision (repeatability)

The repeatability (intraday precision) of the developed method was carried out by determining the binary mixture at three different concentrations for sitagliptin and metformin hydrochloride in bulk using three different concentrations (i.e., 13.36/10, 20.04/12 and 26.72/16 µg mL^{-1} of sitagliptin/metformin hydrochloride, respectively) in triplicates sequentially. The results were reported as %RSD. The low values of %RSD were indicative of the high precision of the method. The %RSD values of the developed method were within the acceptable limit as suggested by the USP and the results are presented in Table 5.

Table 5. Results of repeatability and intraday precision using the developed PLS and PCR models.

Amount taken (actual conc.) (mg mL^{-1})		Predicted conc. (mg mL^{-1})				% Recovery				Acceptable % RSD NMT 2%			
Sita.	Metf.	PLS		PCR		PLS		PCR		PLS		PCR	
		Sita.	Metf.	Sita.	Metf.	Sita.	Metf.	Sita.	Metf.	Sita.	Metf.	Sita.	Metf.
13.36	10	13.31	9.56	13.54	9.76	99.63	95.60	101.35	97.60	0.62	0.58	1.17	0.27
13.36	10	13.33	9.60	13.43	9.80	99.78	96.00	100.52	98.00				
13.36	10	13.18	9.67	13.23	9.81	98.65	96.70	99.03	98.10				
20.04	12	19.90	11.55	20.19	11.70	99.30	96.25	100.75	97.50	0.60	0.38	0.33	0.13
20.04	12	20.08	11.62	20.31	11.73	100.20	96.83	101.35	97.75				
20.04	12	20.13	11.63	20.30	11.72	100.45	96.92	101.30	97.67				
26.72	16	26.22	15.51	25.91	15.65	98.13	96.94	96.97	97.81	1.03	0.40	1.15	0.16
26.72	16	26.65	15.63	26.44	15.70	99.74	97.69	98.95	98.13				
26.72	16	26.15	15.54	25.93	15.67	97.87	97.13	97.04	97.94				

% Recovery = (predicted conc. in µg mL^{-1} /Actual conc. in µg mL^{-1}) ×100.

3.2.3 Accuracy

Accuracy of the method was investigated using standard addition method for three different percentage levels (i.e., 80, 100, and 120%) by recovery experiments. Known amounts of standard solutions containing sitagliptin and metformin hydrochloride were added to sample solutions under investigation to make up solutions of 80, 100, and 120% levels in triplicates and scanned at the range 200–400 nm. The amount of the drugs recovered at each percentage level were determined by using the developed PCR and PLS models. The mean percentage recovery for each percentage level was showed low values of %RSD and the percentage recovery was within the acceptable limit (90–110%) as suggested by the USP. This indicates a high accuracy method at all the three levels and the accuracy data are given in Tables 6 and 7.

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Table 6. Accuracy data of sitagliptin by PCR and PLS models.

%Level	Sample conc. (μg mL^{-1})	Amount of standard sitagliptin (μg mL^{-1})	Total conc. (μg mL^{-1})	Predicted conc. (μg mL^{-1})		%Recovery		%RSD	
				PLS	PCR	PLS	PCR	PLS	PCR
80%	1	9.68	10.68	11.10	10.97	103.91	102.70	0.86	1.08
				10.91	10.89	102.16	101.94		
				10.98	10.74	102.83	100.54		
100%	1	12.10	13.10	13.37	13.36	102.09	101.99	0.87	0.96
				13.28	13.32	101.37	101.69		
				13.14	13.12	100.34	100.18		
120%	1	14.52	15.52	15.45	15.16	99.55	97.66	0.95	0.20
				15.16	15.10	97.67	97.30		
				15.32	15.15	98.69	97.59		

Table 7. Accuracy data of metformin hydrochloride by PCR and PLS models.

%Level	Sample conc. (μg mL^{-1})	Amount of standard metformin HCl (μg mL^{-1})	Total conc. (μg mL^{-1})	Predicted Conc. (μg mL^{-1})		%Recovery		%RSD	
				PLS	PCR	PLS	PCR	PLS	PCR
80%	10	4	14	13.70	13.87	97.86	99.04	0.54	0.35
				13.79	13.93	98.51	99.50		
				13.85	13.96	98.91	99.73		
100%	10	5	15	15.27	15.17	101.79	101.14	0.49	0.23
				15.37	15.22	102.47	101.48		
				15.42	15.24	102.77	101.59		
120%	10	6	16	16.49	16.14	103.04	100.84	0.09	0.09
				16.50	16.11	103.14	100.69		
				16.52	16.11	103.23	100.68		

3.2.4 Specificity (Spiking Method)

The specificity of the method was checked by adding a certain amount of sitagliptin and metformin hydrochloride standard into known amount of marketed sample solution as described in the Methodology section. Specificity data are shown in Tables 8 and 9.

Table 8. Results of specificity for sitagliptin using the developed PCR and PLS models.

Name of marketed sample	Sample conc. (μg mL^{-1})	Amount added (μg mL^{-1})	Total conc. (μg mL^{-1})	Predicted conc. (μg mL^{-1})		%Recovery		%RSD	
				PLS	PCR	PLS	PCR	PLS	PCR
Jauntab	1	12.1	13.1	13.37	13.36	102.09	101.99	0.50	0.21
				13.28	13.32	101.37	101.69		
Jaunmet	1	12.1	13.1	13.14	13.12	100.34	100.18	0.20	0.33
				13.18	13.06	100.63	99.71		
Jauncare	1	12.1	13.1	13.13	12.93	100.22	98.69	0.23	0.70
				13.17	13.06	100.54	99.67		

Table 9. Results of specificity for metformin HCl using the developed PCR and PLS models.

Name of marketed sample	Sample conc. (μg mL^{-1})	Amount added (μg mL^{-1})	Total conc. (μg mL^{-1})	Predicted conc. (μg mL^{-1})		%Recovery		%RSD	
				PLS	PCR	PLS	PCR	PLS	PCR
Jauntab	10	5	15	15.27	15.17	101.79	101.14	0.47	0.24
				15.37	15.22	102.47	101.48		
Jaunmet	10	5	15	15.42	15.24	102.77	101.59	0.06	0.00
				15.40	15.24	102.69	101.59		
Jauncare	10	5	15	15.45	15.25	103.03	101.66	0.42	0.22
				15.55	15.30	103.65	101.97		

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As it can be appeared from these data, recovery for sitagliptin and metformin hydrochloride using the developed PCR and PLS models are within the acceptable limit (90–110%) This suggests that the methods are free from interference due to the excipients used in the commercial formulation.

The above validation results indicate that method is simple, rapid, economical, precise and accurate beside being eco-friendly. Therefor it can be used for a routine analysis in quality control of mixtures and commercial products containing sitagliptin and metformin hydrochloride.

3.2.5 Analysis of marketed formulations

The applicability of the developed methods for the quantification of sitagliptin and metformin hydrochloride in marketed formulations was carried out using the marketed formulation of 50 mg sitagliptin with 500 mg metformin hydrochloride concentration collected from the local pharmacies in the capital Sana'a. Tables 10 and 11 summarized the data obtained for the sitagliptin and metformin hydrochloride in the analyzed marketed formulations.

Table 10. Assay result for sitagliptin and metformin hydrochloride in tablet (marketed sample) by PLS proposed method.

Name of marketed sample	METHOD		PLS					
	Sita.	Metf.	Sita.			Metf.		
	Measured conc. (µg mL^{-1})		Obtained conc. (µg mL^{-1})	%Recovery	%RSD	Obtained conc. (µg mL^{-1})	%Recovery	%RSD
Jauntab	10.68	14	11.10	103.91	1.20	13.70	97.86	0.47
	10.68	14	10.91	102.16		13.79	98.51	
Jaunmet	10.68	14	10.98	102.83	0.50	13.85	98.91	0.24
	10.68	14	10.91	102.11		13.90	99.25	
Jauncare	10.68	14	11.15	104.40	0.26	13.79	98.52	0.44
	10.68	14	11.19	104.79		13.88	99.14	

Table 11. Assay result for sitagliptin and metformin hydrochloride in tablet (Marketed Sample) by PCR proposed method.

Name of marketed sample	METHOD		PCR					
	Sita.	Metf.	Sita.			Metf.		
	Measured conc. (µg mL^{-1})		Obtained conc. (µg mL^{-1})	%Recovery	%RSD	Obtained conc. (µg mL^{-1})	%Recovery	%RSD
Jauntab	10.68	14	10.97	102.70	0.53	13.87	99.04	0.33
	10.68	14	10.89	101.94		13.93	99.50	
Jaunmet	10.68	14	10.74	100.54	0.64	13.96	99.73	0.06
	10.68	14	10.64	99.63		13.98	99.82	
Jauncare	10.68	14	11.17	104.55	0.43	13.75	98.21	0.27
	10.68	14	11.10	103.91		13.80	98.59	

As it can be seen from these data, the sitagliptin and metformin hydrochloride concentrations were within the acceptable limit (90–110%) according to the USP.

3.2.6 Comparing with reference method

Comparison was carried out, with the aid of SPSS program using F-Test to assure non-significant difference between the recovery results of the newly developed methods and that of reference method for both the sitagliptin and metformin hydrochloride. Significance level indicated that null hypothesis was acceptable since the P-value was greater than significance level (Table 12). As for reference methods, sitagliptin and metformin hydrochloride were determined according to the USP as described in the Methodology section.

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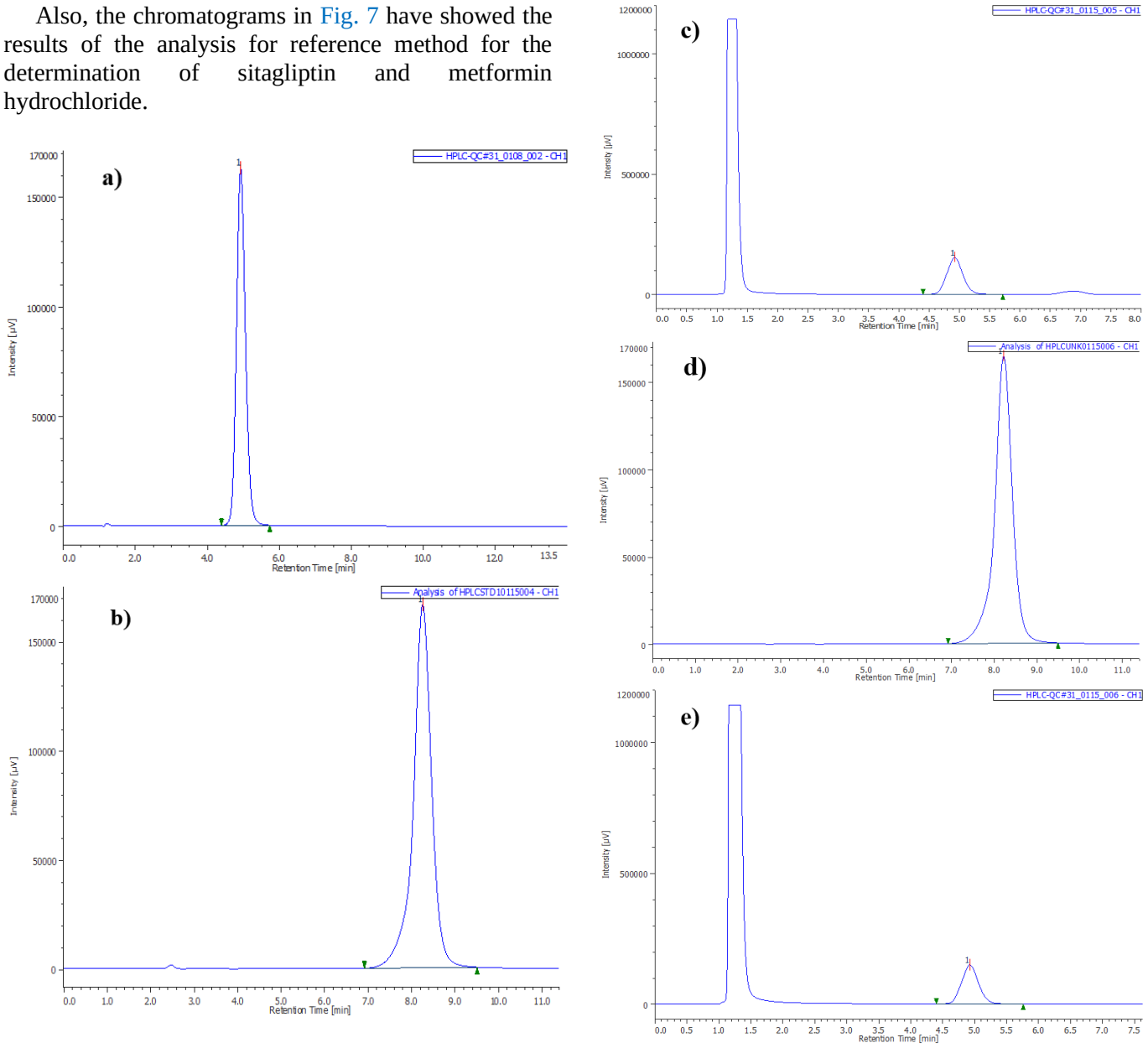
Table 12. Results of statistical comparison between newly developed method and reference method.

Name of marketed sample	Component	Sitagliptin			Metformin HCl		
	Methods	Reference method (HPLC)	PLS	PCR	Reference method (HPLC)	PLS	PCR
Jauntab		101.62	103.91	102.70	96.87	97.86	99.03
		100.86	102.17	101.94	96.94	98.52	99.50
	Mean%	101.24	103.04	102.32	96.91	96.91	98.19
	RSD%	0.53	1.19	0.53	0.05	0.48	0.33
	F-value		0.20	0.18		0.06	0.01
Jaunmet		98.11	102.82	100.54	97.08	98.91	99.73
		98.32	102.00	99.64	98.60	99.25	99.82
	Mean%	98.22	102.47	100.09	97.84	99.08	99.78
	RSD%	0.15	0.49	0.64	1.10	0.24	0.06
	F-value		0.01	0.06		0.25	0.13

F-value at p = 0.01.

Also, the chromatograms in Fig. 7 have showed the results of the analysis for reference method for the determination of sitagliptin and metformin hydrochloride.



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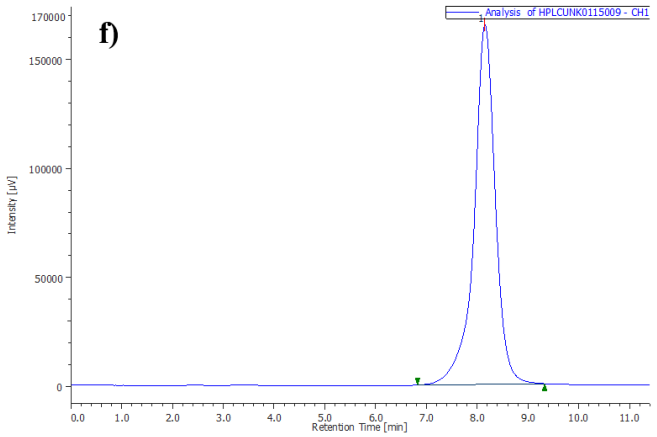

Figure 7. Chromatogram of sitagliptin and metformin HCl standard and commercial samples. **(a)** Standard sitagliptin; **(b)** Standard Metformin HCl; **(c)** Sitagliptin in Jauntab Sample (commercial); **(d)** Metformin HCl in Jauntab Sample (commercial); **(e)** Sitagliptin in Jaunmet Sample (commercial); **(f)** Metformin HCl in Jaunmet Sample (commercial)

4. Conclusions

The proposed chemometrics models (PLS and PCR) has proven to determine simultaneously sitagliptin and metformin HCl in combined mixtures of pharmaceutical dosage forms without excipients interference or each other, and without prior physical separation of the two drugs. Multivariate calibration models were generated using matrices of spectral and concentration data. The validation of the two models and their application to a commercial pharmaceutical dosage form gave excellent results. As a result, the suggested techniques can be applied to regular quality control of the specified medications in their combination dosage form in standard laboratories.

Authors' contribution

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Formal Analysis: Alarbagi, F. A.
Funding acquisition: Not applicable.
Investigation: Alarbagi, F. A.; Al-Maydama, H.
Methodology: Alarbagi, F. A.
Project administration: Almaqtari, M. A.; Al-Odaini, N. A.
Resources: Not applicable.
Software: Alarbagi, F. A.
Supervision: Almaqtari, M. A.; Al-Odaini, N. A.
Validation: Alarbagi, F. A.
Visualization: Al-Odaini, N. A.
Writing – original draft: Alarbagi, F. A.
Writing – review & editing: Al-Maydama, H.

Data availability statement

Data will be available upon request.

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Appendix

Table A1. Results of optimum number of principal factors of sitagliptin for PLS models.

Method	Components to evaluate	Number of components evaluated	Number of components selected		
Cross-validation (Leave-one-out)	Set	10	8		
Model selection and validation for sitagliptin					
Components (pred.)	X Variance	Error	R-sq	Press	R-sq (Pred)
1	0.577399	139.559	0.74980	188.677	0.661736
2	0.999466	1.892	0.99661	2.565	0.995402
3	0.999858	1.036	0.99814	1.568	0.997189
4	0.999888	0.534	0.99904	1.577	0.997172
5	0.999929	0.370	0.99934	1.304	0.997663
6	0.999937	0.116	0.99979	1.390	0.997508
7	0.999946	0.048	0.99991	1.404	0.997482
8	0.999950	0.006	0.99999	1.291	0.997686
9		0.002	1.00000	1.332	0.997612
10		0.001	1.00000	1.335	0.997607

Table A2. Results of optimum number of principal factors of metformin hydrochloride for PLS models.

Method	Components to evaluate	Number of components evaluated	Number of components selected		
Cross-validation (Leave-one-out)	Set	10	8		
Model selection and validation for metformin hydrochloride					
Components (pred.)	X Variance	Error	R-sq	Press	R-sq (Pred)
1	0.743512	14.9504	0.92525	18.7639	0.906180
2	0.999466	0.1424	0.99929	0.1877	0.999062
3	0.999855	0.1157	0.99942	0.1763	0.999119
4	0.999901	0.0742	0.99963	0.1759	0.999120
5	0.999927	0.0394	0.99980	0.1527	0.999237
6	0.999937	0.0143	0.99993	0.1613	0.999194
7	0.999946	0.0059	0.99997	0.1565	0.999217
8	0.999952	0.0023	0.99999	0.1469	0.999265
9		0.0006	1.00000	0.1502	0.999249
10		0.0002	1.00000	0.1523	0.999239

Table A3. The constant and coefficients at each wavelength of sitagliptin and metformin hydrochloride for PLS models.

Sitagliptin				Metformin hydrochloride			
Constant		–1.1712		Constant		0.4045	
Wavelength (nm)	Coefficients	Wavelength (nm)	Coefficients	Wavelength (nm)	Coefficients	Wavelength (nm)	Coefficients
270	–8.554	234.8	0.6532	270	–5.2653	234.8	0.0527
269.8	36.5022	234.6	0.2441	269.8	–3.4147	234.6	0.0577
269.6	–31.8693	234.4	1.2605	269.6	–10.9734	234.4	0.0604
269.4	–25.5432	234.2	0.49	269.4	10.849	234.2	–0.0832
269.2	–65.3233	234	0.1544	269.2	–1.7155	234	0.1432
269	–23.0727	233.8	–0.2522	269	9.9911	233.8	–0.0113
268.8	–11.3679	233.6	–0.5453	268.8	–13.4665	233.6	0.2214
268.6	–5.5928	233.4	–0.344	268.6	4.7408	233.4	0.2054
268.4	–4.1696	233.2	0.8311	268.4	1.394	233.2	0.1359
268.2	–14.1503	233	0.1554	268.2	–4.088	233	0.0633
268	–19.1888	232.8	–0.059	268	–11.7374	232.8	0.1427
267.8	–40.9486	232.6	–0.7372	267.8	–7.8427	232.6	0.0949
267.6	–22.6885	232.4	–0.6594	267.6	–10.4613	232.4	0.1963

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267.4	5.516	232.2	0.6939	267.4	2.0351	232.2	–0.0132
267.2	–50.7754	232	0.6551	267.2	13.406	232	0.0935
267	–9.302	231.8	–0.995	267	1.6967	231.8	0.1395
266.8	–10.1594	231.6	–0.2627	266.8	–2.8888	231.6	0.1843
266.6	18.2208	231.4	–0.5025	266.6	–13.25	231.4	0.1687
266.4	6.7485	231.2	0.0497	266.4	2.8032	231.2	0.1305
266.2	23.5144	231	–0.7094	266.2	0.6477	231	0.1136
266	–9.3416	230.8	0.205	266	–8.4888	230.8	0.0976
265.8	21.6262	230.6	0.152	265.8	2.8843	230.6	–0.2017
265.6	15.7812	230.4	–0.069	265.6	–0.3461	230.4	–0.1925
265.4	36.272	230.2	–0.5146	265.4	4.9661	230.2	0.1252
265.2	23.198	230	–0.9981	265.2	–10.2205	230	0.1216
265	22.0233	229.8	–0.7537	265	–8.7358	229.8	0.0951
264.8	4.3131	229.6	0.0623	264.8	3.7949	229.6	0.4342
264.6	–2.4513	229.4	0.3702	264.6	–1.1286	229.4	–0.077
264.4	16.4876	229.2	–0.4447	264.4	–2.8178	229.2	0.4234
264.2	4.741	229	0.6679	264.2	3.2968	229	0.0984
264	13.294	228.8	0.2232	264	0.8386	228.8	0.3764
263.8	2.2629	228.6	–0.7047	263.8	–1.3713	228.6	0.2014
263.6	–9.7468	228.4	–0.2404	263.6	0.98	228.4	–0.0309
263.4	–13.6458	228.2	–0.671	263.4	8.5777	228.2	0.1123
263.2	13.8386	228	–0.7128	263.2	–4.6343	228	0.1859
263	–1.2977	227.8	–0.4311	263	–4.7391	227.8	0.2667
262.8	–4.2576	227.6	–0.6824	262.8	0.7491	227.6	0.378
262.6	8.4991	227.4	–0.1933	262.6	–6.8955	227.4	0.3419
262.4	10.8477	227.2	–1.2674	262.4	–4.3476	227.2	–0.0296
262.2	4.3527	227	–0.1852	262.2	–7.5522	227	0.1174
262	27.7793	226.8	–0.723	262	2.1918	226.8	0.182
261.8	–16.3076	226.6	–1.5414	261.8	0.0193	226.6	0.2658
261.6	22.6114	226.4	–0.1781	261.6	2.6963	226.4	0.4972
261.4	–4.7696	226.2	–2.6872	261.4	–1.5696	226.2	–0.2154
261.2	–3.511	226	–1.0534	261.2	4.3536	226	0.0702
261	7.9681	225.8	–0.165	261	–3.1001	225.8	0.6035
260.8	41.8407	225.6	–0.2283	260.8	–1.8041	225.6	0.4791
260.6	33.643	225.4	–1.6467	260.6	3.999	225.4	0.5571
260.4	13.2769	225.2	–1.2369	260.4	3.9561	225.2	0.2447
260.2	10.0929	225	–0.5339	260.2	3.1868	225	0.3378
260	9.8518	224.8	–1.3898	260	–2.1213	224.8	0.594
259.8	43.043	224.6	–0.8225	259.8	–1.7967	224.6	0.1454
259.6	5.1607	224.4	–0.6431	259.6	–9.4247	224.4	0.1927
259.4	–34.1511	224.2	–1.1628	259.4	–17.384	224.2	0.3319
259.2	11.5158	224	–1.2444	259.2	–6.5233	224	0.305
259	–14.383	223.8	–0.8308	259	7.4788	223.8	0.4529
258.8	14.5878	223.6	–0.4681	258.8	15.1635	223.6	0.4367
258.6	4.5959	223.4	–0.0295	258.6	3.1614	223.4	0.5613
258.4	–18.8881	223.2	–1.6294	258.4	2.0012	223.2	0.1711
258.2	7.6601	223	–0.7336	258.2	11.4035	223	0.5318
258	4.6961	222.8	–1.8822	258	–5.1932	222.8	0.109
257.8	27.7054	222.6	–1.1367	257.8	10.9597	222.6	0.3162
257.6	0.988	222.4	–1.1225	257.6	10.405	222.4	0.3399
257.4	–12.5001	222.2	–0.8589	257.4	8.7258	222.2	0.3429
257.2	–15.2382	222	–1.6734	257.2	–0.8162	222	0.6074
257	–3.0942	221.8	–0.679	257	1.0974	221.8	0.579
256.8	–4.4602	221.6	0.1903	256.8	–1.5909	221.6	0.1961
256.6	1.7788	221.4	0.089	256.6	–5.2308	221.4	0.23
256.4	27.5921	221.2	–1.1892	256.4	9.6734	221.2	–0.0033
256.2	–18.0223	221	–1.5961	256.2	–2.957	221	0.2731
256	33.2932	220.8	–0.1513	256	8.4664	220.8	0.5852
255.8	–2.1517	220.6	–0.158	255.8	0.3689	220.6	0.3382
255.6	–13.3572	220.4	–0.3497	255.6	7.3895	220.4	0.8263
255.4	–18.1868	220.2	–0.3168	255.4	4.4221	220.2	0.6111

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255.2	26.0096	220	–0.8101	255.2	7.9959	220	0.2208
255	9.8431	219.8	0.0445	255	9.3932	219.8	0.2442
254.8	–27.3872	219.6	–0.9638	254.8	–1.395	219.6	0.1875
254.6	20.7778	219.4	–1.6612	254.6	–2.7202	219.4	0.1146
254.4	–1.2528	219.2	–1.7879	254.4	–4.9913	219.2	0.2444
254.2	–13.5772	219	–1.2254	254.2	–1.6811	219	0.1439
254	–23.6382	218.8	0.3546	254	–4.9847	218.8	0.4906
253.8	–13.4889	218.6	–1.0438	253.8	8.1626	218.6	0.1345
253.6	2.8508	218.4	–0.5056	253.6	1.9851	218.4	0.1787
253.4	23.3554	218.2	–0.4821	253.4	0.8798	218.2	0.131
253.2	–11.656	218	0.9343	253.2	–4.1621	218	0.3666
253	–10.1518	217.8	–0.8597	253	4.5813	217.8	0.6368
252.8	–7.8638	217.6	–1.1187	252.8	–5.3028	217.6	–0.0903
252.6	1.4537	217.4	–0.5789	252.6	–0.1427	217.4	0.1755
252.4	–11.0952	217.2	–0.15	252.4	–1.3668	217.2	0.1429
252.2	–8.0002	217	0.7244	252.2	1.6172	217	0.097
252	2.9909	216.8	–1.5768	252	–4.355	216.8	–0.0646
251.8	13.7498	216.6	0.008	251.8	–4.7497	216.6	0.1884
251.6	–2.0345	216.4	–0.4139	251.6	1.0337	216.4	–0.067
251.4	–9.1847	216.2	–0.4189	251.4	–4.5599	216.2	0.0647
251.2	2.0637	216	–1.2419	251.2	–1.7805	216	0.2227
251	–7.3252	215.8	–0.2577	251	–1.8669	215.8	–0.1081
250.8	0.5645	215.6	1.3402	250.8	0.8755	215.6	0.058
250.6	–4.0354	215.4	0.3949	250.6	1.6955	215.4	0.2037
250.4	–1.5513	215.2	0.5238	250.4	–0.6681	215.2	–0.1017
250.2	6.2078	215	0.249	250.2	–2.0834	215	–0.1637
250	–5.6244	214.8	–0.3013	250	0.1999	214.8	–0.1404
249.8	–5.9114	214.6	1.2798	249.8	–1.3228	214.6	–0.1157
249.6	–3.5399	214.4	–1.4815	249.6	–1.4603	214.4	0.1955
249.4	–1.0941	214.2	1.7537	249.4	0.9512	214.2	0.4072
249.2	–3.1039	214	1.6824	249.2	0.4518	214	0.173
249	–3.6641	213.8	2.1526	249	–0.9982	213.8	0.2943
248.8	–0.5871	213.6	1.8704	248.8	0.8471	213.6	0.2654
248.6	–3.7437	213.4	0.581	248.6	–1.7456	213.4	–0.444
248.4	0.8308	213.2	–1.3783	248.4	0.4118	213.2	–0.1354
248.2	–0.8122	213	0.792	248.2	0.5814	213	–0.1364
248	0.7908	212.8	1.164	248	–0.9923	212.8	0.0905
247.8	–1.3825	212.6	2.2452	247.8	–0.057	212.6	0.131
247.6	2.7731	212.4	0.1635	247.6	–1.3365	212.4	0.0719
247.4	0.6642	212.2	0.4482	247.4	–0.5276	212.2	0.2397
247.2	0.4524	212	0.4644	247.2	0.6951	212	–0.7323
247	0.1823	211.8	0.3519	247	–0.8055	211.8	0.0588
246.8	–0.1467	211.6	2.3998	246.8	–0.0386	211.6	–0.4235
246.6	–2.5052	211.4	2.1745	246.6	–1.6217	211.4	0.1408
246.4	–0.5324	211.2	0.0758	246.4	–1.0455	211.2	–0.0643
246.2	0.4933	211	0.4929	246.2	–0.0042	211	0.3936
246	1.6089	210.8	1.2011	246	–0.5154	210.8	0.2329
245.8	3.8558	210.6	2.4589	245.8	0.5417	210.6	0.0627
245.6	0.8283	210.4	1.5865	245.6	–0.2551	210.4	–0.6584
245.4	0.6869	210.2	1.3387	245.4	–0.7264	210.2	–0.2923
245.2	0.8776	210	1.0135	245.2	–0.4832	210	0.284
245	0.1365	209.8	1.659	245	0.2298	209.8	0.068
244.8	2.305	209.6	–1.298	244.8	–0.3353	209.6	–0.0156
244.6	0.2397	209.4	–1.4035	244.6	–0.2579	209.4	–0.2137
244.4	0.652	209.2	1.3169	244.4	0.0854	209.2	–0.0601
244.2	–0.8754	209	3.0337	244.2	–0.3155	209	–0.1924
244	1.0819	208.8	0.6519	244	–0.2405	208.8	–0.1346
243.8	2.4943	208.6	2.6027	243.8	0.1475	208.6	0.4873
243.6	0.4038	208.4	–0.8664	243.6	–0.3628	208.4	0.6161
243.4	1.1649	208.2	–0.5923	243.4	–0.3957	208.2	–0.9718
243.2	0.9246	208	0.57	243.2	–0.3946	208	–0.324

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243	0.6146	207.8	2.1778	243	–0.2858	207.8	0.1352
242.8	0.0615	207.6	2.404	242.8	–0.4416	207.6	–0.0218
242.6	1.3358	207.4	0.0886	242.6	–0.1321	207.4	0.2638
242.4	2.3687	207.2	–0.2496	242.4	–0.2817	207.2	–0.4744
242.2	0.2692	207	1.8095	242.2	–0.2462	207	0.4651
242	0.515	206.8	2.6284	242	–0.0534	206.8	0.4709
241.8	1.2582	206.6	1.8385	241.8	–0.1296	206.6	–1.4419
241.6	0.5053	206.4	0.7265	241.6	0.1142	206.4	–0.7179
241.4	1.09	206.2	0.1278	241.4	–0.3483	206.2	1.3186
241.2	0.908	206	–2.309	241.2	–0.237	206	–1.4024
241	0.9199	205.8	1.0478	241	0.0217	205.8	0.7645
240.8	–1.2642	205.6	1.0052	240.8	–0.1368	205.6	–0.1055
240.6	0.7109	205.4	–2.4657	240.6	–0.0489	205.4	0.062
240.4	0.3987	205.2	–0.5511	240.4	0.0043	205.2	–0.0275
240.2	1.0164	205	1.2526	240.2	0.0713	205	–0.7603
240	0.5111	204.8	–0.4337	240	–0.0566	204.8	0.0593
239.8	0.5838	204.6	0.3184	239.8	–0.1276	204.6	1.1632
239.6	0.2768	204.4	–1.8168	239.6	–0.2804	204.4	0.4192
239.4	0.1573	204.2	4.7495	239.4	–0.2246	204.2	0.2375
239.2	0.2461	204	–3.6937	239.2	–0.1877	204	–0.5439
239	0.7206	203.8	–1.3017	239	–0.046	203.8	0.4817
238.8	0.7035	203.6	3.4068	238.8	0.237	203.6	–0.1949
238.6	0.7723	203.4	–1.7004	238.6	–0.1562	203.4	0.1481
238.4	1.1926	203.2	1.0425	238.4	0.1137	203.2	0.4436
238.2	0.4957	203	2.7366	238.2	0.1866	203	0.1096
238	0.1993	202.8	0.2214	238	0.0011	202.8	0.9499
237.8	–0.5919	202.6	–0.9995	237.8	–0.1311	202.6	0.005
237.6	–0.2045	202.4	–0.4386	237.6	–0.2809	202.4	–0.1008
237.4	0.2951	202.2	1.5183	237.4	0.0924	202.2	–1.0241
237.2	0.2763	202	3.9352	237.2	–0.0682	202	0.8222
237	0.3454	201.8	–3.9026	237	0.123	201.8	0.185
236.8	0.1578	201.6	–4.6102	236.8	–0.0583	201.6	0.2563
236.6	–0.8305	201.4	–3.1056	236.6	–0.1394	201.4	0.0675
236.4	0.1178	201.2	0.5686	236.4	0.0475	201.2	–0.9555
236.2	0.3159	201	–2.3895	236.2	0.0784	201	0.0037
236	0.6161	200.8	–0.3335	236	0.0782	200.8	–1.1175
235.8	0.9633	200.6	–1.1089	235.8	0.1337	200.6	–0.4999
235.6	0.2546	200.4	0.8255	235.6	–0.0865	200.4	1.5759
235.4	0.3716	200.2	0.2345	235.4	0.042	200.2	–0.4567
235.2	–0.1638	200	2.0276	235.2	0.173	200	1.3505
235	0.409			235	0.0591		

Table A4. Results of the principal components coefficients of sitagliptin and metformin hydrochloride for PCR model.

Mixture No.	Sitagliptin (μg mL^{-1})	Metformin hydrochloride (μg mL^{-1})	Z1	Z2	Z3	Z4	Z5	Z6
1	13.36	8.00	10.84294	2.185204	–0.0434694	0.0576479	–0.0139843	–0.1021771
2	13.36	10.00	12.69862	1.845221	–0.0622337	0.0725021	–0.0115599	–0.1146071
3	13.36	12.00	14.43255	1.621218	–0.0746509	0.0707957	–0.0140199	–0.1112691
4	13.36	14.00	16.44175	1.265448	–0.0755674	0.0880404	–0.0115464	–0.1338847
5	13.36	16.00	18.27641	1.059148	–0.0853648	0.0881577	–0.0111338	–0.1365247
6	16.70	8.00	11.5361	2.966444	–0.0537313	0.0724661	–0.0113965	–0.1132784
7	16.70	10.00	13.4231	2.646652	–0.0779432	0.077781	–0.0093232	–0.1186342
8	16.70	12.00	15.26954	2.319471	–0.0541354	0.0951629	–0.0129627	–0.1177926
9	16.70	14.00	17.10382	2.172555	–0.0758528	0.0893094	0.0015372	–0.1023928
10	16.70	16.00	18.95773	1.866853	–0.0944023	0.0769914	0.0023791	–0.1136455
11	20.04	8.00	12.17808	3.663929	–0.0583895	0.0667501	–0.0093447	–0.102952
12	20.04	10.00	14.02957	3.384557	–0.0636098	0.0895632	–0.0125965	–0.1159239
13	20.04	12.00	15.83709	3.006622	–0.0476592	0.1091312	–0.000479	–0.1135108
14	20.04	14.00	17.75696	2.836101	–0.1076307	0.0624804	–0.013428	–0.119916
15	20.04	16.00	19.77072	2.589099	–0.0620704	0.0841685	–0.0075716	–0.0988977

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16	23.38	8.00	13.05221	4.431188	–0.0777075	0.0878319	–0.009271	–0.118186
17	23.38	10.00	14.89838	4.123749	–0.0764096	0.0853351	–0.0015347	–0.104407
18	23.38	12.00	16.63802	3.798361	–0.0655255	0.1114206	0.0043972	–0.0903928
19	23.38	14.00	18.63537	3.59354	–0.0955316	0.0659751	–0.0306461	–0.106344
20	23.38	16.00	20.41229	3.306786	–0.0370991	0.1190475	–0.0191721	–0.0912103
21	26.72	8.00	13.74611	5.092887	–0.0891428	0.105328	–0.0314232	–0.1326535
22	26.72	10.00	15.43337	4.782102	–0.072408	0.0838503	0.0301399	–0.1550265
23	26.72	12.00	17.35671	4.551464	–0.0895119	0.0670222	–0.0563766	–0.1125212
24	26.72	14.00	18.98121	4.28249	–0.0897928	0.0394045	0.0239509	–0.0873848
25	26.72	16.00	21.00168	3.901466	0.010285	0.0441581	–0.017422	–0.1344944

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