Application of sulfur and nitrogen doped carbon quantum dots as sensitive fluorescent nanosensors for the determination of saxagliptin and gliclazide

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In this study, highly fluorescent sulfur and nitrogen doped carbon quantum dots (S,N-CQDs) were used as fluorescent nanosensors for direct spectrofluorimetric estimation of each of gliclazide (GLZ) and saxagliptin (SXG) without any pre-derivatization steps for the first time. S,N-CQDs were synthesized employing a simple hydrothermal technique using citric acid and thiosemicarbazide. The produced S,N-CQDs were characterized using different techniques including fluorescence emission spectroscopy, UV spectrophotometry, high-resolution transmission electron microscopy and FT-IR spectroscopy. Following excitation at 360 nm, S,N-CQDs exhibited a strong emission peak at 430 nm. The native fluorescence of S,N-CQDs was quantitatively enhanced by addition of increased concentrations of the studied drugs. The fluorescence enhancement of S,N-CQDs and the concentrations of the studied drugs revealed a wide linear relationship in the range of 30.0–500.0 µM and 75.0–600.0 µM with limits of detection of 5.0 and 10.15 µM for GLZ and SXG, respectively.
The proposed method was efficiently used for determination of cited drugs in their commercial tablets with % recoveries ranging from 98.6% to 101.2% and low % relative standard deviation values (less than 2%). The mechanism of interaction between S,N-CQDs and the two drugs was studied. Validation of the proposed method was carried out in accordance with International Conference on Harmonization (ICH) guidelines.

1. Introduction

Gliclazide (GLZ) (N-[[hexahydrocylopenta[c]pyrrol-2(1H)-yl]amino]carbonyl]-4-methylbenzene sulfonamide) (figure 1a) is a second generation sulfonylurea used for the control of type 2 diabetes mellitus (DM) by increasing insulin amount secreted by the pancreatic cells [1]. Previous reports showed that GLZ slows down the progression of diabetic retinopathy. It also has low incidence of hypoglycaemia and good tolerability. Consequently, it is considered as the drug of choice for long-term management of non-insulin-dependent DM patients [1,2].

Saxagliptin (SXG) ((1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile) [3] (figure 1b) is an oral dipeptidylpeptidase-4 inhibitor (DPP-4) which is considered as a novel therapeutic strategy for type 2 DM. It prevents the inactivation of incretins and also stimulates the glucose dependent insulin release [4].

Different methods for estimating GLZ in dosage forms have been reported. These methods include high performance liquid chromatography (HPLC) [5–7], spectrophotometry [2,8–12] and spectrofluorimetry [13–15]. Similarly, SXG was determined using HPLC [16–18], spectrophotometric [19–24] and spectrofluorimetric [20,22,25] methods. The reported spectrofluorimetric methods for GLZ [13–15] and SXG [20,22,25] required prior chemical derivatization reactions using specific reagents; which in turn renders them time consuming and tedious, with a decrease of their greenness profiles. Whereas, in the current study, a green, rapid and simple spectrofluorimetric method was developed for direct estimation of both GLZ and SXG without the need for pre-derivatization steps for the first time. The proposed approach is based on the quantitative enhancement of the sulfur and nitrogen doped carbon quantum dots (S,N-CQDs) native fluorescence upon increasing the concentrations of the studied drugs.

Carbon quantum dots (CDs) are considered as a novel type of fluorescence nanomaterials that range in size from 2.0 to 10.0 nm. They are biocompatible, non-toxic, easily synthesized from cheap starting materials and chemically stable with good photoluminescence properties. Furthermore, they can be easily functionalized using a variety of species and they are highly water soluble [26–29]. For preparation of fluorescent CDs, various approaches have been reported like chemical oxidation [30], microwave-assisted [31], carbonizing organic methods [32] and hydrothermal synthesis [33]. Synthesis of doped CDs is usually carried out by inserting heteroatoms (such as nitrogen (N), phosphorus (P), sulfur (S), fluorine (F), boron (B)) into the CDs’ general structure to enhance their photoluminescence properties [26,34–36]. From literature, CDs were efficiently applied as sensitive probes for the determination of many pharmaceutical compounds in different matrices [26,37,38].

In the present study, a green, simple and economic method was adopted for preparation of S,N-CQDs applying a hydrothermal synthetic approach using thiosemicarbazide (TS) as nitrogen and sulfur source and citric acid (CA) as carbon source producing fluorescence probes for the estimation of each of GLZ and SXG [26].

2. Experimental

2.1. Materials and reagents

GLZ and SXG were obtained from National Organization of Drug Control and Research Center (NODCAR), Cairo, Egypt. Diamicron Tablets (30 mg GLZ/tablet, batch no. (10)29309, Servier Pharmaceutical Co., Cairo, Egypt) and Formigliptin Tablets (5 mg SXG/tablet, batch no. 001087, Multicare Pharmaceutical Co., Cairo, Egypt) were obtained from a local pharmacy in the Egyptian market. CA, TS, sodium acetate, boric acid, glacial acetic acid, methanol and sodium hydroxide were
obtained from Sigma Aldrich (St Louis, MO, USA). Analytical grade chemicals and reagents were used. Double distilled water was used during the study. Different buffers including borate and acetate buffers (0.2 M) were prepared according to the United States Pharmacopoeia (USP) [39].

2.2. Instruments and software

UV-1601 PC spectrophotometer was used to carry out UV spectrophotometric measurements (Shimadzu, Kyoto, Japan) using a 1 cm quartz cell. Agilent Technologies’ Cary Eclipse fluorescence spectrophotometer was used for fluorescence measurements (Santa Clara, USA). The slit width was adjusted to 5 nm and the instrument was set to 750 V mode. Thermo-Fisher Scientific Nicolet—iS10 FT-IR spectrometer was used to obtain the FT-IR spectra (Waltham, MA, USA). It had a 4000–1000 cm$^{-1}$ deuterated triglycine sulfate (DTGS) detector and a Ge/KBr beam splitter. The measurements were taken in 32 scans with a resolution of 4 cm$^{-1}$. A JEM-2100 high-resolution transmission electron microscope (HRTEM) (JEOL, Tokyo) working at 200 kV was used to investigate morphology of S,N-CQDs. pH-meter (Consort, NVP-901, Belgium) was also used.

2.3. Standard stock solutions

GLZ and SXG are relatively insoluble in water so their standard stock solutions (1.0 mM) were prepared in methanol and different concentrations were obtained by serial dilution with double distilled water as appropriate. The prepared solutions were stable for about 10 days when stored at 4°C.

2.4. Synthesis of S,N-CQDs

Synthesis of S,N-CQDs was performed by applying a hydrothermal methodology that was recently reported by Magdy et al. [26]. S,N-CQDs were prepared through mixing of 0.52 g CA and 0.68 g TS with 20 ml of double distilled water, and ultrasonication was carried out for 20 min. The mixture was refluxed at 160°C for 12 h until highly fluorescent S,N-CQDs (dark orange colour) were formed, then cooled and kept in the refrigerator for further use.

2.5. Fluorescence emission spectroscopy

For GLZ, serial concentrations (0, 30.0, 40.0, 50.0, 100.0, 150.0, 200.0, 300.0, 400.0, 500.0 µM) were mixed with 100 µl of S,N-CQDs at pH 7 using 1 ml of borate buffer, and the mixture was heated at 40°C for 10 min. While for SXG, serial concentrations (0, 75.0, 100.0, 150.0, 200.0, 300.0, 400.0, 500.0, 600.0 µM) were added to 150 µl of S,N-CQDs at pH 11 using 1 ml of borate buffer at 25°C for 10 min. Measurement of the fluorescence intensities for both compounds was carried out at 430 nm following excitation at 360 nm. Calibration curves were constructed by graphing each of GLZ and SXG concentrations (in µM) against the difference in fluorescence intensities, then the corresponding regression equations were generated.

Figure 1. Structural formulae of GLZ (a) and SXG (b).
2.6. Quantum yield measurements

The following equation [40,41] was used to calculate the quantum yield of S,N-CQDs:

$$\Phi_{S,N-CQDs} = \Phi_{QS} \times \left( \frac{F_{S,N-CQDs}}{F_{QS}} \right) \times \left( \frac{\eta_{S,N-CQDs}}{\eta_{QS}} \right)^2 \times \left( \frac{A_{QS}}{A_{S,N-CQDs}} \right),$$

where $\Phi$ is the quantum yield, $F$ represents the integrated measured emission intensity, $\eta$ is the solvent refractive index and $A$ is the absorbance.

The standard used was quinine sulfate (QS). It was dissolved in 0.1 M H$_2$SO$_4$ (QY: 0.54 at 350 nm). In the aqueous solutions $\eta_{S,N-CQDs}/\eta_{aq}$ equals to 1.

2.7. Analysis of GLZ and SXG in their tablets

Ten tablets of each of Diamicon or Formiglptin were separately weighed and homogeneously ground. An accurately weighed quantity of the powder corresponding to 30 mg of GLZ or 5 mg of SXG was transferred into a measuring flask (100 ml), followed by addition of 40 ml of methanol. Sonication for 20 min, dilution with methanol to the mark then filtration were performed. Suitable aliquots were transferred from the filtrate into 10 ml measuring flasks, and then the procedure described in §2.5 was performed. The nominal content of tablets was calculated using the corresponding regression equation.

3. Results and discussion

3.1. Characterization of S,N-CQDs

A facile approach was applied in this study to prepare highly fluorescent S,N-CQDs. The adopted approach is based on the hydrothermal treatment of TS as nitrogen and sulfur source and CA as a carbon source [26]. Under UV light, the S,N-CQDs solution exhibited strong blue fluorescence with a long-lasting homogeneous phase and no apparent precipitation for around 14 days in the refrigerator. Spectrofluorimetry, UV absorption spectroscopy, FT-IR and HRTEM were used to characterize S,N-CQDs.

Figure 2A shows the UV absorption spectra of S,N-CQDs, CA and TS. S,N-CQDs had a clear UV absorption band at a maximum wavelength of 330 nm [26,42,43]. Figure 2B also shows the S,N-CQDs fluorescence emission and excitation spectra in aqueous solution. The optimum excitation and emission wavelengths were found to be 360 and 430 nm, respectively. When the excitation wavelength was changed from 340 to 380 nm, the fluorescence spectra of S,N-CQDs shift, and the highest fluorescence intensity was found at 360 nm (figure 2C).

The size and surface morphological characteristics of S,N-CQDs were studied by HRTEM. The samples were placed onto carbon-coated Cu-grid (200 mesh) and investigated using HRTEM at a voltage of 200 kV. As presented in figure 3a, S,N-CQDs are spherical in shape and range in size from 8 to 20 nm.

To study the main surface functional groups of S,N-CQDs, FT-IR analysis was used. As shown in figure 3b, several distinct vibrational modes were observed. The stretching modes of O–H/N–H groups are represented by broad bands at 3500–3100 cm$^{-1}$. C–N vibration is responsible for the band at 2065 cm$^{-1}$. At 1700 cm$^{-1}$, the C=O of the carboxylic acid group is displayed. C=S and C=C are responsible for the stretching maxima of 1233 and 1621 cm$^{-1}$, respectively. A vibration peak was also observed at 578 cm$^{-1}$ for C–H bond [26,44,45].

The quantum yield of S,N-CQDs was also studied as mentioned in §2.6, and they showed a high quantum yield (58.5%) using QS as a reference.

3.2. Interaction mechanism between S,N-CQD and GLZ or SXG

S,N-CQDs emission fluorescence spectra in the presence of various GLZ and SXG concentrations are shown in figures 4 and 5, respectively. By increasing the concentrations of the studied drugs, S,N-CQDs fluorescence intensity was quantitatively enhanced. The native fluorescence of S,N-CQDs was enhanced by 37% and 70% by addition of GLZ (500.0 µM) and SXG (600.0 µM), respectively. The fluorescence enhancement may be attributed to the interaction of each of GLZ and SXG with S,N-CQDs. However, GLZ and SXG cannot form any aggregates with S,N-CQDs, otherwise, the
interaction of GLZ or SXG with S,N-CQDs would lead to decrease of surface defects [46–48]. Moreover, the enhancement of fluorescence intensities may be due to saturation of the dangling bonds at the surface of QDs which effectively removes the QDs surface defects. Due to efficient blocking of non-radiative electron/hole recombination on the surface of QDs, the removal of local trap positions leads to formation of more radiative centres. It seems that the increasing concentrations of GLZ and SXG in the solution get attached to the QDs surface and thereby correct the defective energy levels. This removal of defect levels improves the exciton emissions and subsequently enhances the fluorescence emission intensities of the QDs [46–48].

**Figure 2.** (A) UV absorption spectra of citric acid (a), thiosemicarbazide (b) and S,N-CQDs (c); (B) fluorescence excitation and emission spectra of S,N-CQDs; (C) fluorescence spectra of S,N-CQDs at different excitation wavelengths.
3.3. Inner filter effect of GLZ and SXG

The inner filter effect of the studied drugs was carefully studied to confirm that the enhancement of the fluorescence intensity of S,N-CQDs is due to the interaction between QDs and drugs and not due to the native fluorescence of the studied drugs. It was found that 500.0 µM of GLZ and 600.0 µM of SXG

![Figure 3. (a) The typical HRTEM images of S,N-CQDs; (b) FT-IR spectra of N,S-CQDs.](image)

![Figure 4. Fluorescence emission spectra of S,N-CQDs in aqueous solution upon addition of various concentrations of GLZ (from bottom to top: 0, 30.0, 40.0, 100.0, 150.0, 200.0, 300.0, 400.0, 500.0 µM).](image)
(the maximum concentrations in this study) have neither native fluorescence at 430 nm after excitation at 360 nm nor absorbance at 360 nm, indicating that there was no inner filter effect of either GLZ or SXG (figure 6).

3.4. Optimization of factors affecting interaction of GLZ and SXG with S,N-CQDs

To achieve the maximum sensitivity of the method for GLZ and SXG determination, different factors influencing the fluorescence intensities were studied; including pH of the medium, incubation time and temperature.
3.4.1. Effect of pH

To investigate the impact of pH, the experiments were carried out over pH range from 3.5 to 12 using acetate and borate buffers. It was found that the maximum S,N-CQDs emission intensities were achieved at pH 7 and 11 for GLZ and SXG, respectively (figure 7A). Accordingly, the volume of borate buffer was examined from 0.5 to 4 ml, and it was found that the optimum volume is 1 ml for both drugs.

3.4.2. Incubation time

The incubation time of S,N-CQDs with the investigated drugs was studied by recording the emission fluorescence spectra at time intervals ranging from 1 min to 1 h. The obtained outcomes displayed a fast increasing response after mixing the cited drugs and QDs that reached a constant value after 10 min. The fluorescence signals remained stable for 1 h, giving the suggested method an additional advantage (figure 7B).

3.4.3. Effect of temperature

Over a temperature range of 20–60°C, the influence of temperature on signal enhancing effect of GLZ and SXG was studied. The obtained results demonstrated that the maximum response was achieved at 40°C and 25°C for GLZ and SXG, respectively (figure 7C).

3.5. Method validation

The developed method was validated according to ICHQ2(R1) guidelines [49].

Figure 7. Effect of pH (A), incubation time (B) and temperature (C) on the enhancement of the fluorescence intensity of S,N-CQDs by GLZ (300.0 µM) (a), SXG (200.0 µM) (b).
3.5.1. Linearity and range

The calibration plots were constructed by graphing the concentrations of each drug (µM) versus the difference in fluorescence intensity ($\Delta F = F - F_0$). The linear range was found to be 30.0–500.0 µM and 75.0–600.0 µM for GLZ and SXG, respectively. The regression equations that represent rectangular relationship are as follows:

$$F - F_0 = 0.37C + 20.83 \quad (r = 0.9999) \text{ for GLZ}$$  \hspace{1cm} (3.1)

and

$$F - F_0 = 0.67C + 28.90 \quad (r = 0.9998) \text{ for SXG},$$  \hspace{1cm} (3.2)

where $F_0$ and $F$ are the QDs fluorescence intensities in absence and presence of cited drugs, respectively, $C$ is the concentration in µM. Values of correlation coefficients ($r$) approximating unity indicate the acceptable linearity of the developed procedure (table 1).

3.5.2. LOD and LOQ

The following equations were used to calculate limit of quantitation (LOQ) and limit of detection (LOD) values:

$$\text{LOD} = 3.3 \frac{S_y}{b}, \quad \text{LOQ} = 10 \frac{S_y}{b},$$

where $S_y$ is the standard deviation of the intercept and $b$ is the slope. The obtained results are abridged in table 1.

3.5.3. Precision and accuracy

Three different concentrations of the cited drugs and three replicates of each concentration were used to test the intra-day and inter-day precisions. The obtained results showed small % relative standard deviation (RSD) values (less than 2%), indicating an acceptable precision of the developed approach (table 2). The accuracy and precision were demonstrated by statistical comparison of the obtained results with those given by comparison methods for both drugs [2,23], showing insignificant difference between them as presented by $t$ and $F$ values, respectively [50] (table 3).

3.5.4. Robustness

The method robustness was studied by examining the influence of slight variations in experimental parameters affecting fluorescence intensity of studied drugs. The influence of incubation time (10 ± 1 min) for both drugs, pH (7 ± 0.1) for GLZ and (11 ± 0.1) for SXG, temperature (40 ± 1°C) for GLZ and (25 ± 1°C) for SXG and S,N-CQDs volume (100.0 µl ± 1) for GLZ and (150.0 µl ± 1) for SXG were investigated and showed insignificant impact on % RSD values and % recoveries (table 4).

| parameter | GLZ | SXG |
|-----------|-----|-----|
| linearity range (µM) | 30.0–500.0 | 75.0–600.0 |
| limit of detection (LOD)a (µM) | 5.0 | 10.16 |
| limit of quantitation (LOQ)b (µM) | 15.17 | 30.78 |
| regression equation | $F - F_0 = 0.37C + 20.83$ | $F - F_0 = 0.67C + 28.90$ |
| correlation coefficient ($r$) | 0.9999 | 0.9998 |
| standard deviation (s.d.) | 1.01 | 1.87 |
| percentage relative standard deviation (% RSD) | 1.01 | 1.86 |
| $S_y$, s.d. of the residuals | 0.95 | 3.10 |
| $S_y$, s.d. of the intercept | 0.56 | 2.07 |
| $S_b$, s.d. of the slope | 0.0021 | 0.0061 |

In bold are the limits of detection (LOD) and limits of quantitation (LOQ). The standard deviations (s.d.) are calculated from single replicate analysis. A good correlation coefficient ($r$) is acceptable (above 0.99) for both GLZ and SXG. The LOD and LOQ values are acceptable (less than 0.1 µM) for both GLZ and SXG.

\begin{itemize}
\item aLOD = 3.3 $S_y/b$.
\item bLOQ = 10 $S_y/b$, where $S_y$ is the standard deviation of the intercept and $b$ is the slope.
\end{itemize}
Table 2. Intra-day and inter-day precision data for the determination of the studied drugs by the proposed method. Each result is the average of three separate determinations.

| analyte | conc. taken (µM) | intra-day | | | | inter-day | | |
|---------|----------------|------------|----------|----------|------------|----------|----------|----------|
|         |                 | conc. found ± s.d. (µM) | % RSD | % error | conc. found ± s.d. (µM) | % RSD | % error |
| GLZ     | 100.0           | 101.01 ± 0.93 | 0.92 | 0.53 | 101.11 ± 1.32 | 1.31 | 0.75 |
|         | 200.0           | 197.69 ± 0.86 | 0.87 | 0.5 | 198.82 ± 1.51 | 1.52 | 0.88 |
|         | 300.0           | 302.14 ± 0.98 | 0.97 | 0.56 | 301.21 ± 1.49 | 1.48 | 0.86 |
| SXG     | 100.0           | 101.1 ± 1.1 | 1.09 | 0.63 | 102.07 ± 1.24 | 1.22 | 0.7 |
|         | 200.0           | 196.09 ± 0.63 | 0.65 | 0.37 | 196.81 ± 0.7 | 0.71 | 0.41 |
|         | 300.0           | 293.84 ± 0.36 | 0.37 | 0.21 | 294.3 ± 0.73 | 0.74 | 0.43 |

Within the day.
Three consecutive days.
% Error = % RSD/√n.

Table 3. Application of the proposed method for the determination of GLZ and SXG in pure forms.

| parameter | proposed method | comparison method |
|-----------|----------------|-------------------|
| conc. taken (µM) | % found | mean ± s.d. | Nc | F-value | mean ± s.d. |
| GLZ       | SXG        | GLZ [2]           | SXG [23]        |
| mean ± s.d. | 100.21      | 100.56 ± 2.48     | 99.18 ± 2.35    |
| % RSD     | 1.01        | 1.011             | 1.87             |
| % error   | 0.357       | 0.357             | 0.66             |

| f-value | 5.33 (19)b | 4.74 (19)b |
|         | 0.29 (2.77)b | 0.58 (2.77)b |

Mean of three determinations.
The values between parentheses are the tabulated t- and F-values at P = 0.05 [50].
Number of samples.
## 3.5.5. Selectivity

The proposed method's selectivity was proved by its capacity to estimate the cited drugs in presence of other antidiabetic drugs including metformin, dapagliflozin, empagliflozin, canagliflozin, alogliptin, omarigliptin and glebencamide. The tolerance limits of these drugs were calculated as the concentration that results in 2% relative error [51] and the obtained results are summarized in table 5. In addition, the method selectivity was confirmed by its capacity to analyse the drugs in their tablets with low % RSD values (less than 2%) and high % recoveries (98.64–101.21%); demonstrating no interference from common excipients (table 6).
Due to the high selectivity and reproducibility of the developed method, it was efficiently used to estimate the cited drugs in their commercial tablets with low % RSD values and high % recoveries. Statistical analysis of obtained results with those given by comparison methods for GLZ and SXG [2,23], showed insignificant difference between them regarding precision and accuracy as indicated by the $F$- and $t$-values, respectively [50] (table 6).

### Table 5. Tolerance limits for other antidiabetic drugs in presence of GLZ and SXG.

| Drug             | Tolerance limit with GLZ (µM) | Tolerance limit with SXG (µM) |
|------------------|---------------------------|-----------------------------|
| omarigliptin     | 96                        | 16                          |
| alogliptin       | 13                        | 13                          |
| glebencamide     | 5                         | 17                          |
| metformin        | 15                        | 10                          |
| dapagliflozin    | 111                       | 55                          |
| canagliflozin    | 90                        | 16                          |
| empagliflozin    | 50                        | 23                          |

### Table 6. Application of the proposed method for the determination of GLZ and SXG in tablet dosage forms.

#### GLZ

| Pharmaceutical preparation | Conc. taken (µM) | % found | Conc. taken (µM) | % found |
|----------------------------|------------------|---------|------------------|---------|
| Diamicon tablets (30 mg GLZ/tab) | 30.0             | 99.19   | 30.0             | 100.55  |
|                             | 60.0             | 100.83  | 60.0             | 98.73   |
|                             | 90.0             | 99.73   | 90.0             | 99.94   |
| Mean ± S.D.                 | 99.92 ± 0.84     |         | 99.74 ± 0.93     |
| $F$-value                   | 1.23 (19)$^b$    |         |                  |
| $t$-value                   | 0.25 (2.77)$^b$  |         |                  |

#### SXG

| Pharmaceutical preparation | Conc. taken (µM) | % found | Conc. taken (µM) | % found |
|----------------------------|------------------|---------|------------------|---------|
| Formigliptin tablets (5 mg SXG/tab) | 75.0             | 101.21  | 75.0             | 96.72   |
|                             | 100.0            | 98.64   | 100.0            | 102.54  |
|                             | 150.0            | 100.30  | 150.0            | 98.76   |
| Mean ± S.D.                 | 100.05 ± 1.3     |         | 99.34 ± 2.95     |
| $F$-value                   | 5.14 (19)$^b$    |         |                  |
| $t$-value                   | 0.38 (2.77)$^b$  |         |                  |

$^a$Mean of three determinations.

$^b$The values between parentheses are the tabulated $t$- and $F$-values at $p = 0.05$ [50].
4. Conclusion

In this study, a facile hydrothermal approach was used for synthesis of S,N-CQDs using CA and TS. The prepared S,N-CQDs were characterized using various techniques. S,N-CQDs were used as fluorescent nanosensors for estimation of each of GLZ and SXG depending on their enhancement effect on S,N-CQDs fluorescence intensities. The developed method showed good selectivity for estimation of GLZ and SXG in their tablets and in presence of other antidiabetic drugs. The proposed method is rapid, simple and cost-effective without the need for sophisticated instruments or prior derivatization of the studied drugs. Full validation of the proposed method was performed in accordance with ICH recommendations.

Data accessibility. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.8cz8w9gsm [52].

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References

1. Sweetman SC, Blake P. 2011 Martindale: the complete drug reference. 33. London, UK: Pharmaceutical Press.

2. Panigrahi R, Reheja S, Choudhury PK, Mishra G. 2012 Development of new spectrophotometric method for the estimation of gliclazide. Int. J. Adv. Pharm. Biol. Sci. 2, 124–127.

3. Kim S et al. 2016 PubChem substance and compound databases. Nuclear Acids Res. Spec. Publ. 44, D1202–D1213. (doi:10.1093/nar/gkv951)

4. Augen DJ et al. 2005 Discovery and preclinical profile of saxagliptin (BMS-47718): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. J. Med. Chem. 48, 5025–5037. (doi:10.1021/jm900266p)

5. Geday A, Al-Salami H, Dass CR. 2019 Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. J. Food Drug Anal. 27, 315–322. (doi:10.1016/j.jfda.2018.06.007)

6. Vijayanar S, Palei NN, Katayani T. 2019 Quality by design (QbD) approach to develop HPLC method for estimation of gliclazide and its impurity (gliclazide impurity A) in bulk drug. Curr. Pharm. Anal. 15, 716–723. (doi:10.2174/15734194166681602250932)

7. Shinde GS, Rahul KG, Jadhav R. 2019 Quantitative estimation and validation of metformin hydrochloride and gliclazide in their tablet dosage form by RP-HPLC. JUET Res. J. Sci. Technol. 11, 201–207. (doi:10.5958/2239-2988.2019.00085.S)

8. Jeha B, Salami M. 2019 Spectrophotometric determination of gliclazide in bulk and pharmaceutical formulation using 1, 2-naphthoquinone-4-sulfonic acid sodium salt. Res. J. Pharm. Technol. 12, 5310–5314. (doi:10.5958/0974-360X.2019.00020.X)

9. Sathesh K et al. 2019 Eco-friendly spectrophotometric estimation of gliclazide using hydrotropic solubilization technique. Asian J. Pharm. Anal. 9, 45–48. (doi:10.5958/2231-5675.2019.00010.3)

10. Hasib ST, Taha EA, Eldawy EF, Barakat GH. 2019 Development and validation of spectrophotometric methods for the determination of canagliflozin or gliclazide and metformin in the presence of metformin impurity (1-cyanoguanidine). J. AOAC Int. 102, 1112–1124. (doi:10.5740/jaoacint.18-0234)

11. Marchukova N, Petkova V, Dimitrov M. 2019 UV-spectrophotometric approach in comparative studies of gliclazide modified-release tablets. Bulg. Chem. Commun. 51, 592–595.

12. El-Enany N. 2004 Spectrophotometric determination of gliclazide in pharmaceuticals and biological fluids through ternary complex formation with resin and palladium (II). Farmaco 59, 63–69. (doi:10.1016/j.farmac.2003.08.007)

13. El-Enany N. 2003 Spectrophotometric and spectrophotometric determination of gliclazide in pharmaceuticals by derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole. J. AOAC Int. 86, 209–214. (doi:10.1093/jaoac/86.2.209)

14. Sharma VK, Mazumder B. 2014 Analytical methods for the determination of gliclazide in pharmaceutical formulations and biological fluids. World J. Pharm. Res. 3, 979–997.

15. Szklarek A, Pozycka J, Maciejk-Junczyk M. 2019 Influence of preirradiation on gliclazide—glycated human serum albumin interaction. A spectrofluorometric study. Molecules 24, 111–131. (doi:10.3390/ma20030111)

16. Godje BK, Shinde GS, Joshi S. 2019 Simultaneous estimation and validation of dapagliflozin and saxagliptin in bulk drug and dosage form by RP-HPLC. JUET Res. J. Sci. Technol. 11, 59–63. (doi:10.5958/2239-2988.2019.00008.1)

17. More SS, Sonawane SS, Dhyaj SS, Khirnagar SI. 2018 Development and validation of RP-HPLC method for simultaneous estimation of saxagliptin and dapagliflozin in tablets. Asian J. Pharm. Technol. 8, 145–148. (doi:10.5958/2231-5713.2018.00023.5)

18. Rao BR, Rao VV, Venkateswarlu B. 2019 RP-HPLC method for simultaneous estimation of dapagliflozin and saxagliptin in bulk samples. J. Pharm. Sci. 11, 254–257.

19. El-Bagary RI, Eldawy EF, Ayoub BM. 2012 Spectrophotometric methods based on charge transfer complexation reactions for the determination of saxagliptin in bulk and pharmaceutical preparation. Int. J. Biomed. Sci. 8, 204–208.

20. Moneeb MS. 2013 Spectrophotometric and spectrophotometric methods for the determination of saxagliptin and vildagliptin in bulk and pharmaceutical preparations. Bull. Fac. Pharm. Cairo Univ. 51, 139–150. (doi:10.1016/j.bfpcu.2013.03.003)

21. Lofty H, Mohamed D, Elshahed MS. 2019 Novel univariate spectrophotometric determination of the recently released solid dosage form comprising dapagliflozin and saxagliptin via factorized response spectra: the assessment of current content and dosage unit uniformity of tablets. Spectrochim. Acta A: Mol. Biomol. Spectrosc. 222, 117–120. (doi:10.1016/j.saa.2019.05.025)

22. Abdel-Aziz O, Ayad MF, Tadros MM. 2015 Compatible validated spectrophotometric and spectrophotometric methods for determination of vildagliptin and saxagliptin by factorial design experiments. Spectrochim. Acta A: Mol. Biomol. Spectrosc. 140, 229–240. (doi:10.1016/j.saa.2014.12.102)

23. Shinde BS, Kaliyetti M, Kokane AP. 2020 UV-spectrophotometric method development and validation for estimation of saxagliptin in API and pharmaceutical dosage form. Int. J. Pharm. Res. 12, 63–66. (doi:10.22159/ijpr.2020v12i5.39768)
