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Research Article

O-PHTHALALDEHYDE BASED SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF SITAGLIPTIN IN TABLETS

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ABSTRACT

Objective: The objective of the method was to develop a simple, cost effective and reproducible spectroscopic method for the determination of sitagliptin (STG) in bulk and tablets.

Methods: In this method, sitagliptin reacted with o-phthalaldehyde (OPA) and n-acetyl cysteine (NAC) in a borate buffer (pH 9.8)at room temperature to produce a chromogen. The absorption of reaction product was measured at 338 nm.

Results: The method was linear in a concentration range between 5 and 120µg/ml. The regression line equation was: Y = 0.0140X + 0.0503 with a regression coefficient of 0.9997 (n=6). The limit of detection (LOD) and limit of quantitation (LOQ) were 1.1 and 3.7µg/ml, respectively. The precision was satisfactory; the values of relative standard deviation (RSD) had not exceeded 2%.The average values of recovery study were found to be in the range98.82 - 100.2 ± 0.28 – 1.12%.

Conclusion: The developed method was simple, fast, accurate and precise. It could be applied for routine quality control analysis of STG in tablets.

Keywords: Spectroscopic, Sitagliptin, o-Phthalaldehyde, n-Acetyl cysteine, Tablets.

INTRODUCTION

Sitagliptin (STG) is an oral hypoglycemic agent that blocks selectively the dipeptidyl peptidase 4 (DPP-4) enzyme activities and enhances the body's own ability to lower blood glucose when it is elevated [1,2]. STG is (R)-3-Amino-1-[3-(trifluormethyl)-5,6,7,8-tetrahydro[1,2,4]triazol[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorphenyl)butan-1-one (Fig. 1) [3]. The literature review reveals several methods for determination of sitagliptin in tablets alone or in combination with other hypoglycemic agents. The major reported analytical methods depended on sophisticated instrumental techniques e.g. RP-HPLC [4-9], HPTLC [10], UPLC [11] and capillary zone electrophoresis [12].

Spectrophotometric methods were described either direct [13], after chemical derivatization [14,15], ion pair complexation [16] and charge transfer complexation [17].Some reported spectroscopic methods needed high temperature or included multistep procedure and using organic solvents. Spectrophotometry continues to be very popular, because of its simplicity, versatility, availability in quality control laboratories and low cost. O-phthalaldehyde is a derivatizing agent of primary amines, which was used for analysis of many drugs [18-20].In this study, it was attained to develop a sensitive spectroscopic method for STG analysis which depends on a simple one single step reaction and avoiding vigorous reaction conditions or toxic organic solvent.

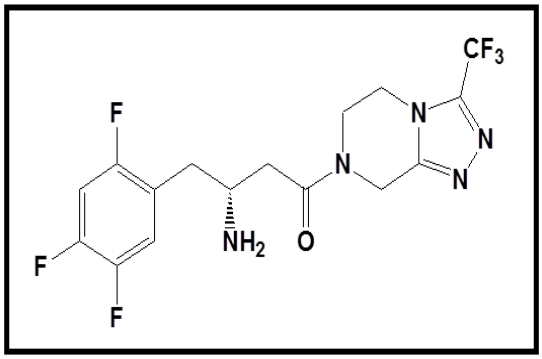

Fig. 1: Structural formula of sitagliptin.

MATERIALS AND METHODS

Apparatus

SHIMADZU UV-1601 spectrophotometer and 1 cm quartz cells were used. Measurements of pH were made with WTWpH 526 Digital pH meter.

Materials

All chemicals used were of analytical grade. Pharmaceutical grade sitagliptin phosphate monohydrate, certified to contain 99.70%, was kindly supplied from Merck Sharp & Dohme Co.(Cairo-Egypt) and film coated tablets Januvia® containing25 mg sitagliptin were obtained from a local pharmacy (Gaza, Palestine). OPA and NAC were purchased from Sigma Aldrich Chemie Gmbh (Munich, Germany).

Preparation of reagents

All the solutions were freshly prepared in distilled water.

OPA solution (0.04%): Prepared by dissolving 40 mg of OPA in 3 ml methanol and dilute with distilled water up to 100 ml. The solution was freshly prepared.

NAC solution (0.07%): Prepared by dissolving 70 mg of NAC in 100 ml volumetric flak with distilled water.

Borate buffer (0.2 M, pH9.8): Dissolve 2.473 g of boric acid into 200 ml volumetric flask with distilled water and adjust pH with 1 M NaOH then complete with water up to the mark [21].

Preparation of stock solution

STG stock solution was prepared by dissolving 100 mg standard substance with distilled water and making the volume to 100 ml in a standard volumetric flask (1mg/ml).

Construction of calibration curve

Different volumes of STG stock solution containing 5-120 µg/ml were transferred into a series of numbered 10 ml volumetric flasks. To each flask 1.0 ml of OPA (0.04%), 0.7 ml NAC (0.07%) and 1.0 ml borate buffer (0.2 M, pH 9.8) were added and mixed gently. The mixtures were allowed to stand at room temperature for 10 minutes and were diluted with distilled water. The absorption was measured at λ 338 nm against blank. The absorbance was measured and

plotted against concentration of STG. The regression line and correlation coefficient were evaluated.

Procedure for STG tablets

Ten Januvia tablets containing 25 mg STG were weighed crushed and their contents mixed thoroughly. A portion of powder equivalent to 100 mg STG active ingredient was accurately weighed and put into 100 ml volumetric flask. About 70 ml distilled water was added and sonicated for 20 min. The volume was made up to the mark with distilled water then filtered with Whatmann filter paper (No 42) to remove insoluble matter. Aliquot from the solution covering the working concentration range was transferred in 10 ml volumetric flask and analyzed as described under construction of calibration curve and analyzed. The results were compared with a reference method.

RESULTS AND DISCUSSION

Determination of absorption maxima (λmax)

STG exhibits λmax at 267 nm. Derivatization of STG can increase the sensitivity and selectivity of a spectroscopic method. STG contains a primary amine group, which can reacts with OPAin the presence of a mercaptan(n-acetylcysteine) at room temperature in basic mediato produce isoindole derivative (Fig. 2) [22]. The spectrum of the derivative (STG/OPA/NAC) was recorded against blank, which exhibited a red shifted λmaxat 338 nm (Fig.3).

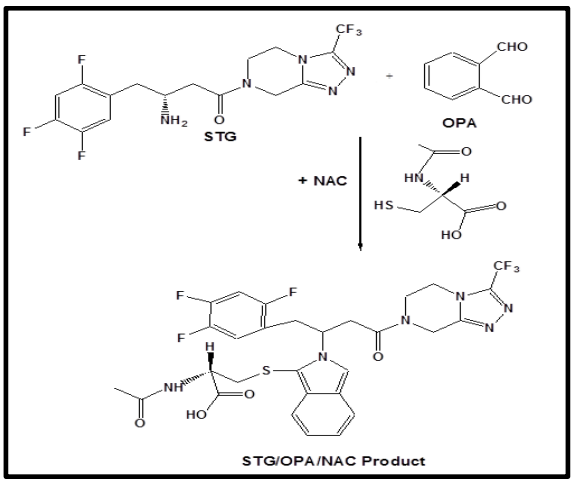

Fig. 2: A proposed reaction of STG with OPA/NAC [22].

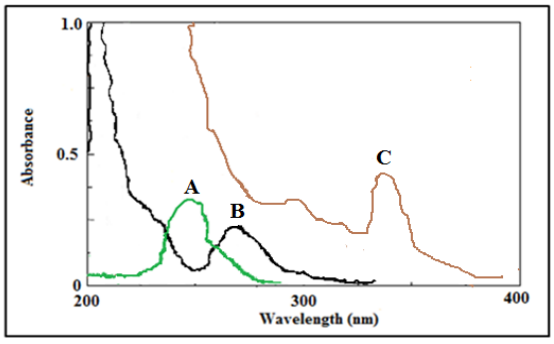

Fig. 3: Absorption spectra (A) blank against water (B) STG (20µg/ml)against water (C) the reaction product of STG (20 µg/ml)with OPA/NAC against blank.

Optimization of reaction conditions

Effect of OPA concentration

To study the effect of OPA concentration and volume on the reaction OPA solutions ranged from 0.01 to 1.5% (1 ml) were used as described in construction of calibration curve and the absorbance were measured. High absorption readings were attained in the range 0.04-1.2%, beyond which the absorbance slightly decreased. Different volumes (0.5–2.0 ml) were also tested. An OPA concentration of 0.04% (1 ml) was used in the subsequent experiments.

Effect of NAC solution

The reaction of OPA with primary amine required an SH-donor compound like NAC. The advantages of NAC are the stability of its product with OPA/primary amine and lack of unpleasant free thiolsodor [23-26]. Different concentrations of NAC solution (0.01-2.0%) were tested. The maximum absorbance was achieved when NAC concentration 0.07% and 0.7 ml volume.

Effect of pH

To optimize the reaction the pH effect of different buffers (borate, bicarbonate and phosphate), strengths (0.1-1.0 M) and volumes (0.5-5.0 ml) were tested. The buffers have a pH ranged from 8.0 to 11.5, since the reaction proceeds in alkaline media [22]. A borate buffer (0.2 M, pH 9.8) and 1.0 m volume were adequate (Fig. 4).

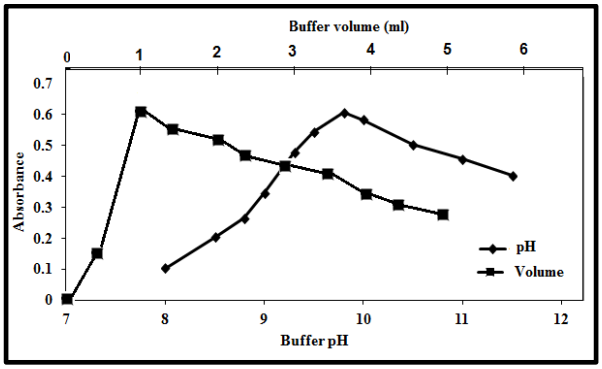

Fig. 4: Effect of buffer pH and volume of buffer on the absorbance of reaction product for STG (20µg/ml) with OPA/NAC.

Effect of OPA/NAC molar ratio

Changing the molar ratio of OPA to NAC additive (0:1 - 5:1) was studied at STG concentration 30 µg/ml. A 1:1 molar ratio (Fig.5) was satisfactory and resulted in the highest absorbance measurement.

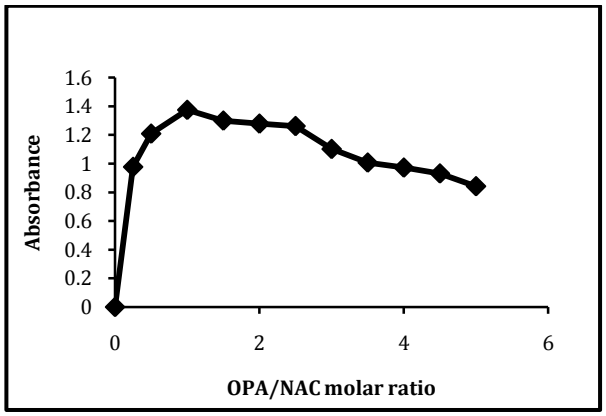

Fig. 5: Effect of OPA/NAC mole ratio on derivatization reaction.

Effect of temperature and time

A completion of the reaction was achieved at room temperature (25 ± 2°C), within 10 min. High temperature up to 60 °C has affected the reaction negatively, which can be due to instability of the reaction adduct.

Effect of diluting solvent

Different solvents (water, methanol, ethanol, isopropanol, acetonitrile, acetone and methyl acetate)were tested. Upon diluting with water a transparent solution was obtained indicating the

solubility of reaction product and the absorbance was high. Water was used as diluting solvent which has the advantage of avoiding organic solvents.

Stability of STG/OPA/NAC chromogen

Measuring the absorbance of reaction product at different time intervals up to 4 hrs after dilution revealed that it was stable up to 60 min (Fig. 6). This allowed the processing of large batches of samples with comfortable measurements.

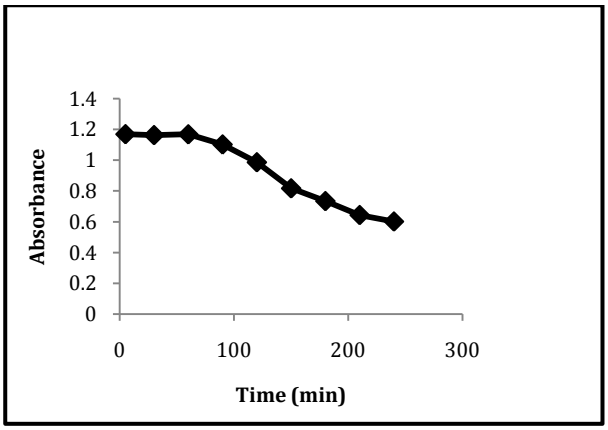

Fig.6: Stability of STG/OPA/NAC chromogen.

Method validation

The developed method was validated according to ICH guidelines [27]. It included linearity, range, LOD, LOQ, accuracy, precision, robustness and ruggedness.

Linearity and limits of detection and quantitation

The calibration graphs(n=6) were obtained by plotting the absorbance values versus STG concentrations. The linearity was calculated using the least square regression model. The regression line equation was Y = 0.0140X + 0.0503 (r=0.9997) where Y is the absorbance at 338 nm and X is the concentration of STG in µg/ml in the range 5-120 µg/ml, and r is the correlation coefficient. Other important statistical parameters are summarized in Table 1. The molar absorptivity (ε) was $5.1*10^5$ $L.mol^{-1}.cm^{-1}$. The LOD and LOQ were calculated according to ICH guidelines [27] and found to be 1.1 and 3.7 µg/ml, respectively.

Table 1: Quantitative parameters of the developed method

(n = 6)

Parameter	STG
Linear range (µg/ml)	5-120
Molar absorptivity ε (L/mol.cm)	$5.1*10^5$
LOD (µg/ml)	1.1
LOQ (µg/ml)	3.7
Correlation coefficient (r)	0.9997
Slope	0.0140
Intercept	0.0503
Standard deviation of intercept	$5.2*10^{-3}$
Standard deviation of slope	$3.5*10^{-4}$
Standard deviation of residuals	$1.8*10^{-3}$

Accuracy and precision

To determine accuracy of the method known quantities (50%, 100%, and 150%) of STG standard were added to pre-analyzed drug preparation of 3 different concentrations (5, 20, 40 µg/ml). The percent recovery ranged 98.82 - 100.20 ± 0.28 – 1.12% (Table 2). Recovery studies indicated that the method is specific, since excipients normally present in tablets have not interfered with the developed method. Intra- and inter-day precision were evaluated by analyzing prepared samples of STG three times per concentration (10, 50 and 80 µg/ml) on three consecutive days. Precision was expressed as relative standard deviation (%RSD) and the results are given in Table 3. The proposed method was adequately accurate and precise.

Table 2: Recovery studies for determination of STG by the developed method

Pre-analyzed STG(µg/ml)	Added STG (µg/ml)	Recovery(% ± SD)[a]
5	2.5	98.82 ± 0.62
	5.0	99.91 ± 1.12
	7.5	99.94 ± 0.31
20	10.0	100.02 ± 0.50
	20.0	100.10 ± 0.28
	30.0	99.95 ± 0.93
40	20.0	100.11 ± 0.34
	40.0	100.20 ± 1.0
	60.0	99.88 ± 0.97

[a]: Values were the mean of three determinations.

Table 3: Intra- and inter-day precision of the developed method

STG concentration (µg/ml)	RSD (%)	
	Intra-day (n =3)	Inter-day (n =3)
10	1.21	0.97
50	0.86	0.73
80	0.51	0.63

Robustness and ruggedness

The robustness of the developed method was tested by examining the influence of small variations of the reaction conditions; these include the buffer pH, reagent concentration, reagent volume and reaction time on its analytical performance.

In each case only one parameter was changed while all other conditions were kept constant. Percentage of recovery was calculated in each case (Table 4). It was found small variations had not affected significantly the developed method indicating its reliability during routine analysis; recovery values were 98.51-101.03 ± 0.06-1.42%.

Ruggedness was assessed by using the same operational conditions to analyze 3 different STG concentrations (10, 50, 80 µg/ml) by using two different instruments Shimadzu UV 1601, Japan and PerkinElmer lambda 25, England) at two different laboratories and two different days. Results obtained from lab-to-lab and day to day variations were reproducible as the RSD did not exceed 2% (Table 5).

Table 4: Robustness of the developed method

Parameter	Recovery (%± SD)[b]
Recommended conditions[a]	100.12 ± 0.14
Buffer pH	
9.5	99.15 ± 0.11
10.0	99.88 ± 0.52
Buffer volume (ml)	
0.8	100.31 ± 0.06
1.2	98.74 ± 0.46
OPA concentration (%w/v)	
0.03	99.69 ± 0.87
0.05	100.20 ± 0.31
OPA volume (ml)	
0.8	100.07 ± 1.06
1.2	100.11 ± 0.13
NAC concentration (%w/v)	
0.06	98.51 ± 1.15
0.08	99.03 ± 0.97
NAC volume (ml)	
0.5	99.76 ± 0.12
0.9	99.45 ± 1.42
Reaction time (min)	
8	101.03 ± 0.32
12	99.99 ± 0.13

[a]: Conditions were mentioned under construction of calibration curve, [b]: Values were the mean of three determinations.

Table 5: Ruggedness of the developed method

STG concentration (µg/ml)	RSD (%)[a]	
	Shimadzu UV 1601	PerkinElmer lambda 25
10	1.30	1.18
50	0.53	0.42
80	0.21	0.26

[a]: Values were the mean of three determinations

Application of the developed method for determination of STG in tablets Januvia tablets were subjected to analysis of their STG content by the developed OPA method alongside with a reference method [13]. The label claim percentage was 99.58± 0.81. This result was compared with that obtained from the reference method by statistical analysis with respect to the accuracy by *t*-test and precision by F-test (Table 6). No significant difference was found at 95% confidence level providing similar accuracy and precision in the determination of STG by both methods.

Table 6: Determination of STG in tabletsby OPA and a reference method

Dosage form	Recovery (%± SD)[a]		t-value[b]	F-value[b]
Januvia®	OPA method	Reference method[c]		
	99.58±0.81	100.84±0.43	0.48	2.01

[a]:Values were the mean of five determinations,[b]: Theoretical valuesat 95% confidence limit, t= 2.306, F = 6.388, [c]: Reference method [13].

OPA versus other spectroscopic methods

OPA assisted spectroscopic method for determination of STG in bulk and tablet was compared with other published spectroscopic methods.

The important parameters of comparison are listed in table 7. The linear range obtained per OPA method was advanced compared with described methods [13, 15].

The sensitivity was enhanced and coincidentally heating and organic solvent were avoided in OPA method in comparison to procedure based on NQS and picric acid [14].

Charge transfer complexes [17] showed a red shifted λmax, however the sensitivity was low and the reaction proceeded in organic solvents. Although ion-pair complexation [16] exhibited comparable sensitivity and linearity to OPA method, it is a multistep process and needed extraction with chloroform.

Table 7: Important parameters of spectroscopic and OPA method for determination of STG

Spectroscopic Method	λmax (nm)	Range(µg/ml)	Molar absorptivity(L/mol.cm)	LOD(µg/ml)	LOQ(µg/ml)	Ref.
Direct	267	5-40	$0.27*10^2$	0.139	0.422	13
AA &FA[a]	430	5-25	$1.067*10^4$	1.947	5.9	15
NQS[b]	450	25-150	$0.27*10^4$	-	-	14
PA[c]	420	5-25	$1.4*10^4$	-	-	14
DDQ[d]	461	50-300	-	9.79	29.67	17
PC[e]	555	50-900	-	1.4	4.25	17
TCNQ[f]	837	20-120	-	3.26	9.89	17
BTB[g]	412	25-125	$1.028*10^4$	-	-	16
BCG[h]	419	10-50	$1.08*10^4$	-	-	16
OPA[i]	338	5-120	$5.1*10^5$	1.1	3.7	-

[a]: Reagents were acetyl acetone and formaldehyde, [b]: 1,2-Naphthaquinone-4-sulfonic acid sodium salt, [c]: Picric acid, [d]: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, [e]: p-Chloranil, [f]: 7,7,8,8-Tetracyanoquinodimethane,[g]:Bromothymol blue, [h]:Bromocresol green, [i]: o-Phthalaldehyde.

CONCLUSIONS

The present work described a validated spectroscopic method for determination of STG in tablets after being derivatized with OPA/NAC in alkaline media. The proposed method was rapid, accurate, specific, and precise. It has the advantages of avoiding expensive instrumentation, heating, multistep procedure and toxic organic solvents. The method can be conveniently used in quality control laboratories.

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