Available online at www.pharmacie-globale.info ISSN 0976-8157

Research Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

# SPECTROPHOTOMETRIC DETERMINATION OF SITAGLIPTIN AND METFORMIN IN THEIR PHARMACEUTICAL FORMULATION

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Received: 28 March 2012; Revised: 26 April 2012; Accepted: 30 April 2012; Available online: 5 May 2012

## ABSTRACT

Two simple, accurate and precise spectrophotometric methods were developed and validated for the determination of sitagliptin phosphate monohydrate (STA) and metformin hydrochloride (MTF). The first method was based on measuring the absorbance of STA at 268 nm in the range of 25-500 µg $mL^{-1}$. The second method was the isosbestic point method. The total mixture concentration was calculated by measuring the absorbance at 257 nm. The proposed methods used to determine each drug in binary mixture. The results were statistically compared using one-way analysis of variance (ANOVA). The developed methods were satisfactory applied to the analysis of the pharmaceutical formulation and proved to be selective and accurate for the quality control of the cited drugs in their pharmaceutical formulation.

**Keywords:** metformin; sitagliptin phosphate; spectrophotometry.

## INTRODUCTION

Sitagliptin is [(2R)-1-(2,4,5-trifluorophenyl)-4-oxo-4-[3-(trifluoromethyl) -5,6dihydro [1,2,4] triazole [4,3-a] pyrazin -7(8H)-yl]butan-2-amine], (figure **1**). It is an orally active and selective inhibitor of dipeptidyl peptidase-IV that is used for the treatment of Type 2 diabetes.[1]

Metformin hydrochloride is (*N*,*N*-dimethylimidodicarbon imidic diamide hydrochloride) (figure **2**). It is prescribed as an oral hypoglycemic agent, used in the management of non-insulin dependent diabetes mellitus.[2]

**Figure 1. Chemical structure of sitagliptin phosphate (STA)**

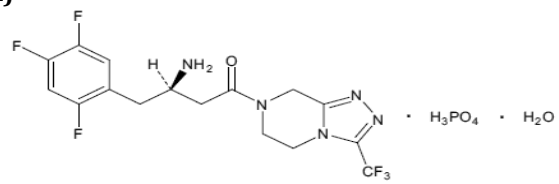
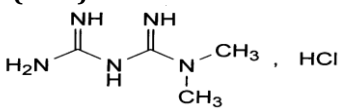


**Figure 2. Chemical structure of metformin hydrochloride (MTF)**



The literature survey reveals several analytical methods for quantitative estimation of sitagliptin[3] in combinations[4,5] metformin[6-9] and in other combinations.[10-15]

In modern analytical laboratories there is always a need for simultaneous determination of STA and MTF in the drug analysis. The present work aimed to develop simple and sensitive spectrophotometric methods for selective quantification of STA and MTF in their pure forms or even in their pharmaceutical formulation. The methods described here include direct spectrophotometry and isosbestic point methods.

## MATERIALS AND METHODS

### Instruments

Spectrophotometer: shimadzu UV-1601 PC, dual-beam UV-Vis spectrophotometer (Japan), with matched 1-cm quartz cells, connected to an IBM-compatible PC and an HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software version 3.7 was used to process the absorption and the derivative spectra. The spectral bandwidth was 2 nm with wavelength-scanning speed of 2800 nm $min^{-1}$.

### Chemicals and reagents

All chemicals and reagents were analytical–grade, and water was always bi-distilled. Reference sitagliptin phosphate (STA) standard was kindly donated by Merck & Co., Inc., Whitehouse station (New jersey, USA) Its purity was found to be 98.87% (n=6), according to a reference spectrophotometric method.[16] Reference metformin hydrochloride (MTF) standard was kindly supplied by Cid Co. (Cairo, Egypt). Its potency was found to be 99.39 (n=6), according to the USP official method.[17] Janumet® tablets BN: 0426580, labeled to contain 50 mg STA as phosphate salt and 1000 mg MTF as hydrochloride salt. De-ionized water: Bi-distilled from "Aquatron" Automatic Water still A4000 was provided by Bibby sterillin Ltd. (UK).

### Standard solutions

STA standard solution (1mg $mL^{-1}$) and MTF (0.1mg $mL^{-1}$) standard solution in distilled water. All calculations and samples preparation of STA and MTF for reference material and pharmaceutical formulation were done on basis of the salt form. Solutions were freshly prepared on the day of analysis and stored in refrigerator to be used within 24 h.

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**Direct spectrophotometric method**

**Spectral characteristics of STA and MTF:** Two aliquots (3 mL) of STA (1mg $mL^{-1}$) and (1.5 mL) of MTF (0.1mg $mL^{-1}$) aqueous solutions were separately transferred into two 10-mL volumetric flasks. The volumes were completed with distilled water to obtain final concentration of 300 µg $mL^{-1}$ of STA and 15 µg $mL^{-1}$ of MTF. The spectra of the prepared solutions were recorded separately.

**Linearity:** Portions equivalent to (0.25-5 mL) of STA standard stock solution (1 mg $mL^{-1}$) were separately transferred into a series of 10-mL volumetric flasks. Each flask was completed to the volume with distilled water to reach the concentration range of 25-500 µg $mL^{-1}$.

The absorption spectra of each solution was recorded against distilled water as a blank at 268 nm, and then plotted against its corresponding concentration and the regression parameters were computed and used for its determination of unknown samples.

**Isosbestic method**

**Construction of the calibration curve:** Aliquots from STA and MTF stock solutions (1 mg $mL^{-1}$) and (1& 10 mg $mL^{-1}$) equivalent to 25–500 µg of STA and 20-1000 µg of MTF were separately transferred into two sets of 10 mL volumetric flasks and completed to the mark with distilled water. The spectra were recorded for both drugs using distilled water as a blank, then the absorbance was measured at 268 nm for STA and 257nm (Aiso) for MTF. Two calibration curves were constructed for each drug relating the absorbance at the selected wavelength to the corresponding drug concentration and the regression equations were computed.

For 2 drugs, 1 and 2, the absorbance can be calculated at any wavelength (λ) from the equation:

$$A = A^{1\%}_{1cm} b\, c \quad \text{.....(1)}$$

Therefore, for drug 1:

$$A_1 = A_1{}^{1\%}_{1cm} C_1 \quad \text{.....(2)}$$

and for drug 2:

$$A_2 = A_2{}^{1\%}_{1cm} C_2 \quad \text{.....(3)}$$

Where $A_1$ and $A_2$ are the absorbence of drug 1 and drug 2, respectively; $C_1$ and $C_2$ are the concentrations of drug 1 and drug 2, respectively; $A_1{}^{1\%}_{1cm}$ and $A_2{}^{1\%}_{1cm}$ $_{1cm}$ are the absorbtivities when the path length (b) is 1 cm and concentration is 1 g/100 mL for drug 1 and drug 2, respectively. If $C_1 = C_2$, $A_1 = A_2$, and $b_1 = b_2$, this λ is called the isosbestic point, and at this λ:

$$A_1{}^{1\%}_{1cm} = A_2{}^{1\%}_{1cm}$$

For a mixture of both drugs, the absorbance at this λ can be calculated from the equation:

$$A_M = A_1{}^{1\%}_{1cm} C_{1M} + A_2{}^{1\%}_{1cm} C_{2M}$$

$$A_M = A^{1\%}_{1cm}(C_{1M} + C_{2M}) = A^{1\%}_{1cm}(C_{TM})$$

Where $A_M$ is the absorbance of their mixture at isosbestic point and $C_{1M}$ and $C_{2M}$ are the concentrations of drug 1 and drug 2 in the mixture, respectively. Where $C_{1M}$ and $C_{2M}$ are the concentrations of drug 1 and drug 2 in the mixture, respectively, and $C_{TM}$ is the concentration of their mixture.

Therefore we can conclude that:

$$(C_{1M} + C_{2M}) = (C_{TM})$$

Thus, having the total concentration of both drugs, if the concentration of one of them can be determined separately by any other method, the concentration of the second drug can be calculated by subtraction. This theory can be confirmed experimentally by recording the absorbance spectra of a certain concentration of the 2 drugs and the absorbance spectra of a binary mixture containing the same concentration.

Separate aliquots equivalent to 500 µg $mL^{-1}$STA and 500µg $mL^{-1}$ MTF were transferred from their stock solutions each (1 mg $mL^{-1}$) into two 10-mL volumetric flasks, then completed to volume with distilled water to get final concentration of 50 µg $mL^{-1}$STA and 50µg $mL^{-1}$ MTF . A binary mixture was prepared by transferring aliquots equivalent to 250 µg $mL^{-1}$ STA and 250µg $mL^{-1}$ MTF into a 10-mL volumetric flask and then completing volume with distilled water to get final mixture of 25 µg $mL^{-1}$ STA and 25µg $mL^{-1}$ MTF . The spectra of the prepared solutions were recorded separately.

**Analysis of laboratory prepared mixtures**

The absorbance of the laboratory-prepared mixtures containing different ratios of STA and MTF were measured at 268 nm corresponding to the contents of STA only, and at 257 nm ($A_{iso}$) corresponding to the total content of STA and MTF in the mixture. The concentration of STA alone and the total concentration of the two drugs were calculated from their corresponding regression equations. The actual concentration of MTF in each mixture was obtained by subtraction of STA concentration from the total concentration.

**Assay of pharmaceutical formulations (Janumet® tablets)**

Twenty tablets were weighed from dosage form and the average weight was calculated, tablets were crushed to furnish a homogenous powder and 2.375 gm of powdered tablets were dissolved by the aid of an ultrasonic bath for 2 hours, filtered through Whatman no.41 filter paper to prepare stock solutions of the drugs as described under each method.

## RESULTS AND DISCUSSION

**Direct spectrophotometric method**

STA can be determined directly at 268 nm without any interference from MTF (direct absorbance) (Figure **3**). A linear relationship was obtained in the range of 25-500 µg $mL^{-1}$ for STA (Figure **4**).

**Figure 3. absorption spectra of STA 300 µg (---) and MTF 15 µg (—— )**

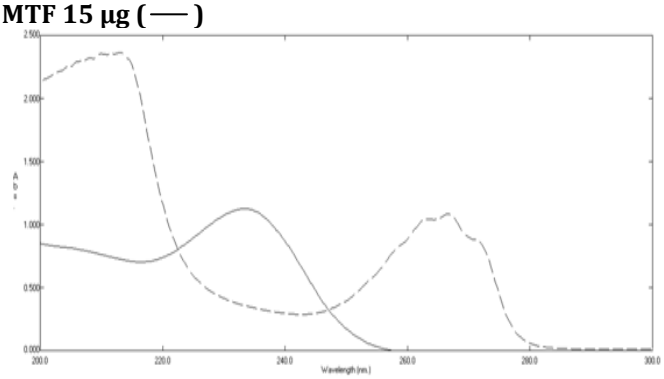
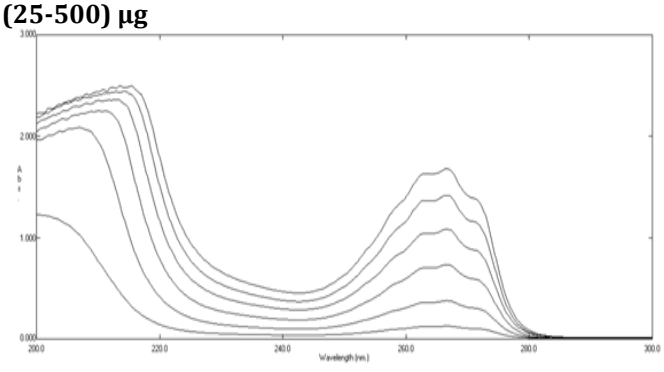


**Figure 4. Scanning profile of STA calibration curve (25-500) µg**



The corresponding regression equation was computed and found to be:

A = 0.0034 C + 0.053

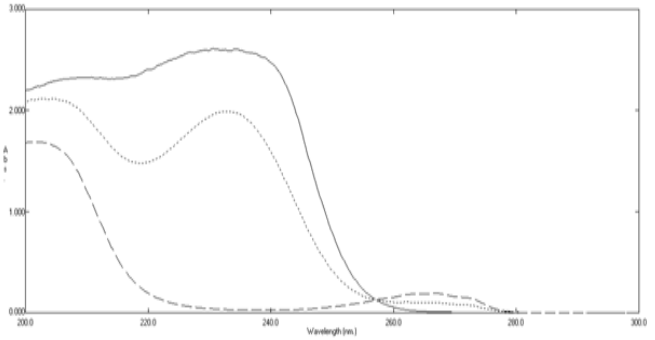
(r=0.9997), at 268 nm

Where, A is the absorbance of STA at 268 nm, C is the concentration of STA (µg $mL^{-1}$) and r is the correlation coefficient. The precision of the proposed method was confirmed and the mean percentage recoveries were found to be 99.64 ± 0.991 % at 268 nm.

### Isosbestic spectrophotometric method

Erram and Tipnis[19] developed the isosbestic spectrophotometric method. The method was used for the simultaneous determination of MTF and STA in their binary mixtures. At the isosbestic point the mixture of drugs acts as a single component and gives the same absorbance value as pure drug. Thus, by measuring the absorbance value at the chosen isosbestic point, 257 nm (Aiso) (Figure **5**), the total concentration of both STA and MTF could be calculated, while the concentration of STA in MET and STA mixture could be calculated, without any interference, at 268 nm. Thus the concentration of MTF could be calculated by simple subtraction.

**Figure 5. Scanning profile of STA 50 µg (---), MTF 50 µg (....) and binary mixture of 25 µg of MTF and 25 µg of STA (—)**



A linear correlation was obtained between the absorbance values against the corresponding concentrations of both drugs at their corresponding wavelengths. The characteristic parameters of the regression equation of isosbestic method for the determination of MTF and STA were given in table **3**:

$A_{iso}$ = 0.0022 C + 0.0299

r = 0.9993 at 257 nm for total MTF and STA

A = 0.0034 C + 0.053

r = 0.9997 at 268 nm for STA

Where $A_{iso}$ is the absorbance at isosbestic point 257 nm, A is the absorbance at 268 nm, C is the concentration of the drug in µg $mL^{-1}$ and r is the correlation coefficient.

### Statistical analysis

Results of the suggested methods for determination of STA and MTF were statistically compared with those obtained by applying the reported methods[16] and[17], respectively. The suggested methods were successfully applied for the determination of STA and MTF in laboratory prepared mixtures with good precision as shown in (table **1**).

**Table 1. Detemination of sitagliptin and metformin in laboratory prepared mixtures**

| Methods | Direct spectrophotometric method | Isosbestic point method |
|---|---|---|
| Sitagliptin Mean ± S.D. | 101.56±1.032 | ------- |
| Metformin Mean ± S.D. | -------- | 101.23±0.847 |

The proposed methods were also used for estimating the concentration of both drugs in their pharmaceutical formulations as shown in (table **2**).

**Table 2. Determination of sitagliptin and metformin in janumet® tablets**

| Preparation Janumet® tablets BN: 0426580 | Direct spectrophotometric method | Isosbestic point method |
|---|---|---|
| Sitagliptin, Mean ± S.D. | 100.68±0.612 | ---------- |
| Metformin, Mean ± S.D. | ---------- | 98.50±0.262 |

Assay parameters and a validation sheet for determination of the studied drugs are shown in (table **3**).

**Table 3. Assay parameters and validation sheet for determination of sitagliptin and metformin**

| Parameter | Direct spectrophotometric method | Isosbestic point method |
|---|---|---|
| | STA | MTF |
| Range | 25-500 µg $mL^{-1}$ | 20-1000 µg $mL^{-1}$ |
| Slope | 0.0034 | 0.0022 |
| Intercept | 0.053 | 0.0333 |
| Mean | 99.64 | 100.61 |
| S.D. | 0.991 | 1.464 |
| Variance | 0.983 | 2.143 |
| Coefficient of variation | 0.995 | 1.455 |
| Correlation coefficient (r) | 0.9997 | 0.9999 |
| R.S.D.(%)[a] | 1.214-1.473 | 1.578-0.914 |
| R.S.D.(%)[b] | 0.902-0.453 | 1.388-1.3655 |

***a*** *the inter-day (n=6) relative standard deviation of 50-100 µg for STA by direct spectrophotometric method and for MTF by isosbestic method.*

***b*** *the intra-day (n=6) relative standard deviation of 50-100 µg for STA by direct spectrophotometric method and for MTF by isosbestic method.*

The calculated t- and F-values[18] were found to be less than the corresponding theoretical ones, confirming good accuracy and excellent precision (table **4**).

**Table 4. Statistical comparison for the results obtained by the proposed methods and reference methods for the analysis of sitagliptin and metformin**

| Parameter | Direct spectrophotometric method | Isosbestic point method | Reference method(16) | Official USP method(17) |
|---|---|---|---|---|
| | STA | MTF | STA | MTF |
| Mean | 99.64 | 100.61 | 99.87 | 99.30 |
| S.D. | 0.991 | 1.464 | 0.954 | 1.20 |
| Variance | 0.983 | 2.143 | 0.91 | 1.44 |
| n | 7 | 12 | 6 | 6 |
| f-test | 1.080( 4.95 ) | 1.488( 4.74 ) | -- | -- |
| Student's t-test | 0.312(2.201) | 2.026( 2.120 ) | -- | -- |

[a] *the values in the parentheses are the corresponding theoretical t-and F-values at p = 0.05 [18].*

## CONCLUSION

It can be concluded that the proposed procedures are simple and do not require either sophisticated techniques or instruments. They are also sensitive, selective and can be used for the routine analysis of the cited drugs in their available dosage forms. The methods are also suitable and valid for application in laboratories lacking liquid chromatographic instruments. The reagents used in the proposed methods are cheap and readily available. The procedures applied in each method do not involve any

critical reactions or tedious sample preparations also no need for derivatization procedures. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility of assaying STA and MTF in their pharmaceutical formulation without any interference from the excipients. There is no significant difference between the proposed method and the reported methods [16] and [17] and could be applied for routine analysis of pure drugs or in its pharmaceutical formulation.

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