

# Colorimetric Determination Of Sitagliptin As An Oxidation Derivative Of Ninhydrin

Ali Khalil Mahmood<sup>1</sup>, Taghreed N-A Omar<sup>2</sup>, Khalid Waleed S. Al-Janabi<sup>1,\*</sup>

<sup>1</sup> Chemistry Department, College of Education for Pure Sciences/ Ibn Al-Haitham, University of Baghdad, Baghdad 10053, Iraq.

<sup>2</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq

\* Correspondent author Author: [khalid.w.s@ihcoedu.uobaghdad.edu.iq](mailto:khalid.w.s@ihcoedu.uobaghdad.edu.iq)

## ABSTRACT

A sensitive and accurate colorimetric method was developed for the determination of the Sitagliptin phosphate monohydrate, here and after will be named Sitagliptin, in its pure and pharmaceutical form. The suggested approach is based on boosting the sensitivity of the traditional spectrometric methods by derivatizing Sitagliptin into a colored product that absorbs the visible spectrum at 573 nm.

The proposed method has effectively improved the sensitivity and the limit of detection for the analysis of Sitagliptin.

A linear calibration curve was obtained over the concentration range of 0.1-10 µg/ml with a correlation coefficient of 0.9983. The calculated recovery was within the range of 98.98 – 100.11%. While the limit of detection LOD and the limit of quantification LOQ were 0.03 mg/L and 0.1 mg/L respectively.

The established procedure has been validated as exhibited linear relationship of spectroscopic absorbance intensity versus analyte concentrations and can be applied for routine quantification of the active constituent of Sitagliptin in different pharmaceuticals.

**Keywords:** Sitagliptin, Sitagliptin Phosphate Monohydrate, ninhydrin, diabetes, Colorimetric determination

## Correspondence:

Ali Khalil Mahmood

1 Chemistry Department, College of Education for Pure Sciences/ Ibn Al-Haitham, University of Baghdad, Baghdad 10053, Iraq.

\*Corresponding author: Ali Khalil Mahmood email-address:

[khalid.w.s@ihcoedu.uobaghdad.edu.iq](mailto:khalid.w.s@ihcoedu.uobaghdad.edu.iq)

## INTRODUCTION

Sitagliptin, as a phosphate monohydrate, figure (1), is used with a proper eating regimen, scheduled workouts and perhaps with other medications to control high blood glucose. It is utilized in individuals with diabetes type-2 (Aschner et al., 2006). Controlling high blood sugar prevents kidney damage, poor eyesight, nerve problems, loss of polyps, and problems with the sexual ability (Bakris et al., 2020; Landon et al., 2020).

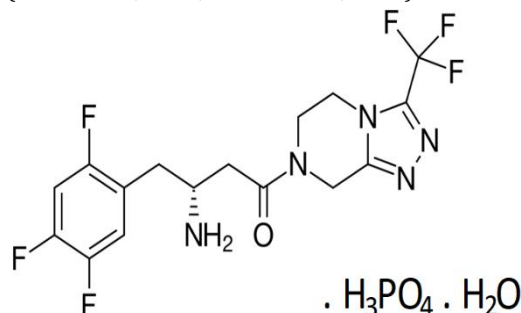


Figure 1. Structure of Sitagliptin phosphate monohydrate

As with COVID-19 fatalities, diabetes is a significant risk factor for adverse effects (Bloomgarden, 2020). However, a recent study has found that treatment with Sitagliptin exhibited a reduction in the mortality rate in patients with type-2 diabetes and COVID-19 (Solerte et al., 2020). Sitagliptin Phosphate was quantized spectrometrically in the ultraviolet region as a pure compound as well as pharmaceutical bulk (Pathade, Pawar, Gaikwad, & Panhalkar, 2011). In another study, a spectrophotometric method has developed and validated the first-order derivative of the UV spectrum for the quantification of Sitagliptin in pharmacological formulations (Pritam et al., 2011). Likewise, Sitagliptin has been simultaneous analyzed in the presence of metformin using laser diode thermal desorption connected to tandem mass spectrometry (Swales, Gallagher, Denn, & Peter, 2011).

Nevertheless, a sensitive voltammetric approach was reported for the quantification of Sitagliptin using Renewable Amalgam Film Electrode (Górska, Paczosa-Bator, & Piech, 2020). Furthermore, a developed method of “high-performance liquid chromatography-tandem mass spectrometry” (HPLC-MS/MS) was implemented for the detection of Sitagliptin in human plasma (Loh et al., 2020). Likewise, liquid chromatography was used successfully for a simultaneous determination of anti-diabetic compounds (Shokouhi, Sohrabi, & Mofavvaz, 2020). Nevertheless, a major privilege of spectrometric methods over most of the other analytical techniques is their cost affordability and high sensitivity (Al-Janabi, Mahmood, & Luaibi, 2020).

This study has developed a new colorimetric approach for the determination of Sitagliptin in pharmaceutical preparations depending on the usage of ninhydrin as an oxidation reagent for the aliphatic primary amine existing on the structure of the Sitagliptin molecule (Gorog, 2018; Rahman & Azmi, 2001). It was fast and has an excellent sensitivity toward the estimation of Sitagliptin and can be implemented for routine determinations.

## Instruments and Materials

- UV/Vis double beam spectrophotometer, Shimadzu 1800, Japan.
- Sitagliptin phosphate monohydrate, Beijing Sjar Technology Development Co., Ltd., China.
- Ninhydrin (powder), Hubei Aoks Bio-Tech Co., Ltd., China.
- Januvia (100 mg) tablets, MSD Pharmaceuticals Pvt Ltd, “Merck Sharp & Dohme Corp.”, Kenilworth, N.J., U.S.A.

## RESULTS & DISCUSSION

A stock solution of 1000 ppm Sitagliptin phosphate monohydrate was prepared by dissolving 100 mg of the drug (Molar mass 523.32 g/mol) in 10ml of methanol

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then the volume completed with distilled water to the mark of a 100 ml volumetric flask.

A set of standard solutions (10, 20, 40, 60, 80 ppm) was prepared from the stock solution and has examined

spectrometrically. Two distinguishable absorption peaks were found at 205nm and 267nm as shown in figure (2). However, the sensitivity, as well as the detection limit, were not good enough.

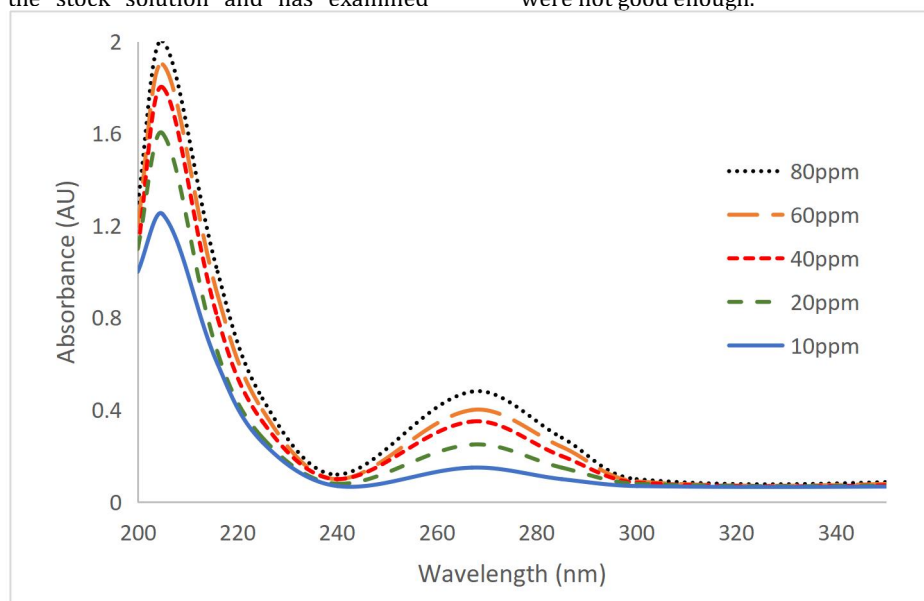


Figure (2). UV Spectrum of Sitagliptin aqueous solution

The sensitivity and hence the detection limit were boosted by derivatization. As the aliphatic primary amino group in Sitagliptin was reacted with ninhydrin to produce a colored derivative to shift the absorbance to the visible area. The reaction took place by adding the reagent of ninhydrin to the standard aqueous solution of Sitagliptin and by heating the mixture in a water bath for 18 min at 70 °C. The resultant brownish-purple solution was measured at 573 nm.

Optimizing the reaction conditions was examined by changing one variable at a time and measure its influence as a function for the absorbance intensity of the colored product.

The time of derivatization reaction was very critical and should be precisely controlled. The highest color intensity has been obtained after heating the mixture of the drug with the ninhydrin for 17 min. The color persists until 20 min. However, heating the mixture beyond 20 min decreases the color intensity.

Reaction temperatures lower than 65 °C did not give the required color intensity. While reaction temperatures higher than 80 °C made the color change very fast and hard to control.

The ninhydrin concentration (Molar mass 178.14 g/mol) was estimated on the basis that its molar mass is about one-third of the molar mass of the drug molecule. Thus, the addition of 0.5 ml of ninhydrin standard solution in the concentration of 10 mg/L to 2.5 ml of the analyte solution will be enough to react with all the molecules of the drug. Stoichiometry of the oxidation reaction has been studied following Job's method of continuous Variation (Likussar & Boltz, 1971). The molar ratio of Sitagliptin vs ninhydrin was about 1:2.

Ninhydrin, as an oxidizing reagent, was expected to oxidize the primary amino group of Sitagliptin causing it to be eliminated and replaced by the reduced ninhydrin molecule which results in a colored product absorbs at 573 nm (Figure 3).

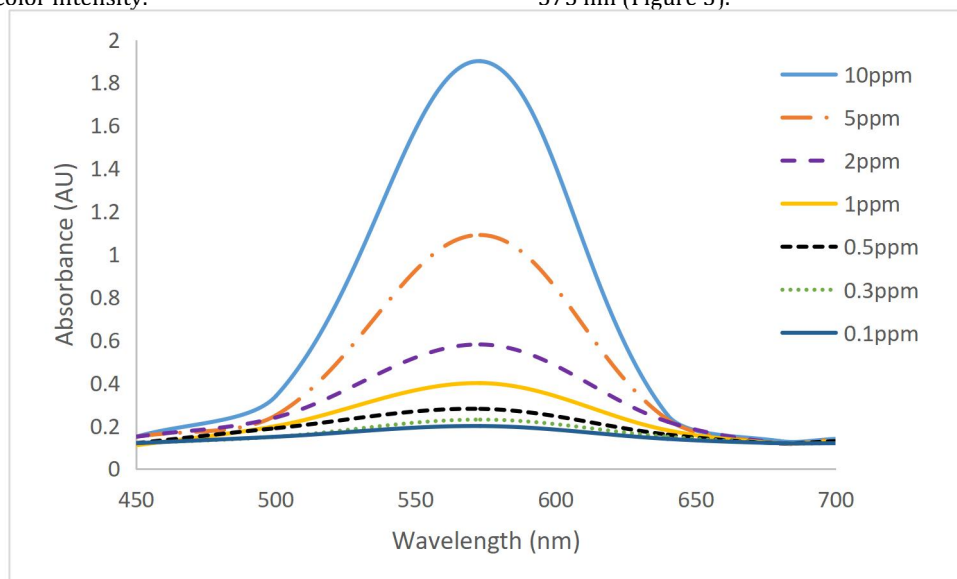


Figure (3). UV/Vis Spectra of the colored derivative produced from the reaction of ninhydrin reagent with different concentrations of Sitagliptin

A calibration curve was built upon obtaining the optimized conditions for the oxidative reaction of Sitagliptin with ninhydrin (Figure 4).

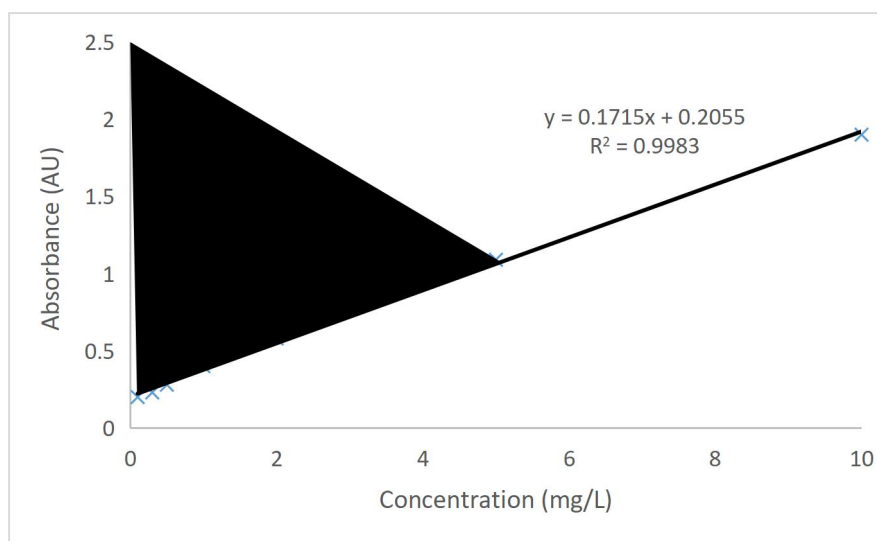


Figure (4). Calibration curve of the reaction product of Sitagliptin with ninhydrin at 573 nm

The limit of detection LOD can be estimated from the linear equation of the calibration curve or the mean absorption value of the blank multiplied by three ("ICH Official web site: ICH," n.d.). Hence the LOD was 0.03 mg/L. While the limit of quantification LOQ can be gotten from the mean absorption value of the blank multiplied by ten and it was 0.1 mg/L.

The pharmaceutical product known as Januvia from MSD Pharmaceuticals was subjected to analysis following the newly suggested procedure. The recovery has been calculated and was found to be 98.98 – 100.11%.

For validation, the linearity, accuracy, and precision of the method were studied. A series of calibration standards of different concentrations were analyzed. The linear regression equation was attained for the proposed procedure. The calibration curve showed linear dependence of the absorbance and obedience to Beer's law. However, the accuracy and precision of the method were determined by analyzing each standard solution in five replicates. The relative standard deviation (RSD) was found to be 1.03% which is very satisfactory.

#### CONCLUSION

The proposed procedure was eco-friendly, cost-effective, and sensitive for spectrometric quantification of Sitagliptin in its pharmaceutical formulations. The approach was highly reproducible with an RSD of around 1%. Yet, it can be applied to other pharmaceutical preparations. Method accuracy and precision were determined with five replicates of Sitagliptin for individual tests and consequently, it is appropriate for routine assessment of Sitagliptin.

#### References

1. Al-Janabi, K. W. S., Mahmood, A. K., & Luaibi, H. M. (2020). Development of a simple colorimetric determination of dexamethasone. *International Journal of Drug Delivery Technology*, 10(2), 255–258. <https://doi.org/10.25258/ijddt.10.2.12>
2. Aschner, P., Kipnes, M. S., Lunceford, J. K., Sanchez, M., Mickel, C., & Williams-Herman, D. E. (2006). Effect of

- the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. *Diabetes Care*, 29(12), 2632–2637.
3. Bakris, G. L., Agarwal, R., Anker, S. D., Pitt, B., Ruilope, L. M., Rossing, P., ... Joseph, A. (2020). Effect of Finerenone on Chronic Kidney Disease Outcomes in Type 2 Diabetes. *New England Journal of Medicine*.
4. Bloomgarden, Z. T. (2020). Diabetes and COVID-19. *Journal of Diabetes*, 12(4), 347–348. <https://doi.org/10.1111/1753-0407.13027>
5. Gorog, S. (2018). *Ultraviolet-Visible Spectrophotometry in Pharmaceutical Analysis*. New York, U.S.: CRC Press.
6. Górska, A., Paczosa-Bator, B., & Piech, R. (2020). Highly Sensitive Adsorptive Stripping Voltammetric Method for Sitagliptin Determination on Renewable Amalgam Film Electrode. *Journal of The Electrochemical Society*, 167(13), 136510.
7. ICH Official web site: ICH. (n.d.). Retrieved November 3, 2020, from <https://www.ich.org/page/quality-guidelines>
8. Landon, R., Gueguen, V., Petite, H., Letourneur, D., Pavon-Djavid, G., & Anagnostou, F. (2020). Impact of astaxanthin on diabetes pathogenesis and chronic complications. *Marine Drugs*, 18(7), 357.
9. Likussar, W., & Boltz, D. F. (1971). Theory of continuous variations plots and a new method for spectrophotometric determination of extraction and formation constants. *Analytical Chemistry*, 43(10), 1265–1272.
10. Loh, G. O. K., Wong, E. Y. L., Tan, Y. T. F., Lee, Y. L., Pang, L. H., Chin, M. C., ... Peh, K. K. (2020). Simple and rapid LC-MS/MS method for determination of sitagliptin in human plasma and application to bioequivalence study. *Journal of Chromatography B*, 1159, 122337.
11. Pathade, P., Pawar, A., Gaikwad, A., & Panhalkar, A. (2011). Development and Validation of Stability Indicating UV Spectrophotometric Method for the Estimation of Sitagliptin Phosphate in Bulk and Tablet Dosage Form. *Journal of Pharmacy Research*, 2(4), 596–602.

12. Pritam, J., Amar, C., Bhargav, D., Shani, P., Santsaran, P., & Hiren, S. (2011). Development and validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in Formulation. *International Journal of Drug Development & Research*, 3(4), 194–1999.
13. Rahman, N., & Azmi, S. N. H. (2001). Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations. *Il Farmaco*, 56(10), 731–735.
14. Shokouhi, S., Sohrabi, M. R., & Mofavvaz, S. (2020). Comparison between UV/Vis spectrophotometry based on intelligent systems and HPLC methods for simultaneous determination of anti-diabetic drugs in binary mixture. *Optik*, 206(January), 164304. <https://doi.org/10.1016/j.ijleo.2020.164304>
15. Solerte, S. B., D'Addio, F., Trevisan, R., Lovati, E., Rossi, A., Pastore, I., ... Bellante, R. (2020). Sitagliptin Treatment at the Time of Hospitalization Was Associated With Reduced Mortality in Patients With Type 2 Diabetes and COVID-19: A Multicenter, Case-Control, Retrospective, Observational Study. *Diabetes Care*.
16. Swales, J. G., Gallagher, R. T., Denn, M., & Peter, R. M. (2011). Simultaneous quantitation of metformin and sitagliptin from mouse and human dried blood spots using laser diode thermal desorption tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 55(3), 544–551. <https://doi.org/10.1016/j.jpba.2011.02.030>