Research Article

Spectrophotometric Method for the Determination of Vildagliptin in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

New, simple, sensitive, accurate, selective and cost effective method has been developed for the determination of Vildagliptin in bulk and in pharmaceutical dosage forms. The developed method , is a chemical derivatization method involving proton transfer from 2,4,6-trinitrophenol (picric acid) as Lewis acid to the secondary amino group of Vildagliptin as Lewis base in chloroform to form a yellow charge transfer complex exhibiting maximum absorption (λ_{max}) at 410 nm. The method was linear in the concentration ranges 3-32 µg/ml with correlation coefficient (R^2= 0.9994), intra-day, inter-day precision (as RSD) and accuracy were determined. The results obtained were statistically validated .The percentage recovery and placebo interference show that the method was not affected by the presence of common excipients. The method was applied to the determination of Vildagliptin in tablets. The percentage recovery was 97.018± 0.591(n=5).The results obtained were statistically compared to those obtained from a reference method. The advantages of the proposed method were discussed.

Keywords: Charge transfer reaction, Chemical derivatization, Picric acid, Spectrophotometer, Vildagliptin.

INTRODUCTION

Vildagliptin, ((2S)-1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile), is an oral hypoglycemic drug of the dipeptidylpeptidase-4 (DPP-4) inhibitor class.[1]

DPP-4 inhibitors represent a new therapeutic approach to the treatment of diabetes mellitus type 2. They inhibit the inactivation of incretins allowing to stimulate the release of glucose dependent insulin[2] (i.e. increase insulin secretion and lower glucagon secretion).[3] Vildagliptin is not official in any pharmacopeia yet. Literature survey reveals that a few Spectrophotometric, spectrofluorimetric 1, 4 and HPLC methods[5,6] were reported for its determination in bulk and pharmaceutical formulations as a single component and simultaneously with metformin[7-9] in dosage forms.

Among the various methods available for the quantitation of drugs, spectrophotometry continues to be the most convenient analytical technique, because of its simplicity, accuracy, low cost and wide availability in most quality control laboratories.

Due to the poorly absorbing Chromophores in the examined drug (λ_{max} 203nm, molar absorptivity ε=7810)[4], its direct measurement of ultraviolet absorption is susceptible to potential interferences from common excipients or impurities.

Picric acid has been used as a Derivatizing reagent in the development of Spectrophotometric methods for the determination of many pharmaceuticals.[10-12] Vildagliptin is bearing a secondary amino group (Figure 1)and is a potential candidate for the reaction with picric acid based on charge transfer complex formation.[13-15] In the present

investigation, a new simple, rapid, accurate, and cost effective visible Spectrophotometric method has been developed for the estimation of Vildagliptin as raw material and in tablet dosage form.
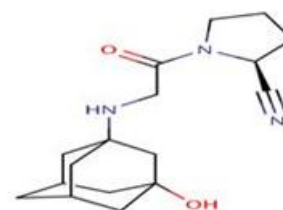

Figure 1: Chemical structure of Vildagliptin

Materials

Picric acid (2,4,6-trinitrophenol) (Pure 99.8%, Merck, Germany).

Chloroform (analytical grade, pure 99%, Needham market. Suffolk England).

Pharmaceutical grade Vildagliptin, certified to contain 99.90% was kindly supplied from the National Drug Quality Control & Research Labs, Ministry of Health (Damascus-Syria).

Vildagliptin tablets nominally containing 50mg Vildagliptin per tablet, were purchased from local market (Biomed Vildagliptin, manufactured by Biomed for pharmaceutical industry, Syrian Arab Republic, batch number T14023).

Apparatus

Cecil, Ce7200 double beam UV-Visible recording spectrophotometer with pair of 1 cm matched quartz cell was used to measure absorbance of solutions.

Ultrasonic, Branson 200.

- A SHIMADZU analytical balance with 0.01 mg.

Standard solution and reagent

Vildagliptin stock solution (1 mg/mL) was prepared by dissolving 50 mg of drug to 50mlchloroform.

Stock solutions were protected from light and kept refrigerated at 4C°. The stock solution was further diluted with same solvent to obtain working solution of concentration 60µg/ml.

A Solution of 0.1% w/v reagent was freshly prepared by dissolving 50 mg picric acid in 50 ml chloroform.

Procedure for pure drug

Aliquots of the working standard solution of Vildagliptin were transferred in a series of 10 ml volumetric flask to give final concentrations of 3-32µg/ml. 1 ml of picric acid (0.1%w/v) was added to each flask and volume was made up to the mark with chloroform. The solutions were shacked well and allowed to stand for 5 minutes to complete the reaction. The absorbance was measured at 410 nm against blank reagent prepared similarly. The drug concentration was calculated from the corresponding regression equation of the calibration graph.

### Procedure for commercial tablets

Twenty tablets of labeled claim 50 mg of Vildagliptin were weighed accurately. An average weight of each tablet was determined. An accurately weighed quantity of powder equivalent to100 mg of Vildagliptin was transferred into 100ml volumetric flask and sonicated for 10 min with40 ml of chloroform then made up to the mark with same solvent. The contents were mixed well then filtered rejecting the first portion of filtrate. The prepared solution concentration is 1000 µg/ml. A 6 ml of 1000 µg /ml solution was further diluted to 100 ml with chloroform to give final concentration of 60µg/ml. Aliquots of the tablets solution were treated as under the general recommended procedures for the reaction with picric acid. Then the concentration of the drug was calculated using calibration curve.

RESULTS AND DISCUSSION

Absorption spectra

Vildagliptin shows maximum absorption at 203 nm using chloroform as a solvent (Figure 2). The reaction is between picric acid as Lewis acid[15,16] (electron acceptor) and Vildagliptin as electron donor, at the same time the proton of the hydroxyl group of picric acid will transfer to the secondary amine of Vildagliptin. Molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge-transfer complexes which absorb radiation in the visible region.[17]

The absorption spectra of the yellow colored products were recorded at 300–600 nm against the corresponding blank solution. The resulted product showed maximum absorbance at 410nm as shown in (Figure 2)
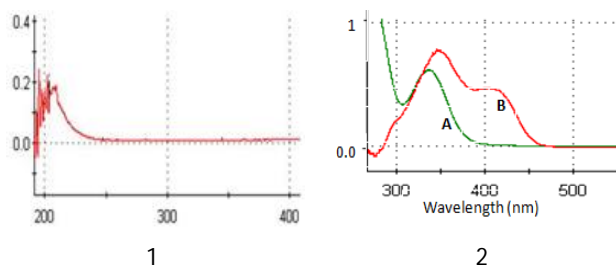

Figure 2: Vildagliptin spectra using chloroform as a solvent (1) Absorption Spectra of (A) reagent blank against chloroform and (B) Vildagliptin- reagent complex against reagent blank (2).

It was found, that absorbance increases with increasing picric acid concentration and reached its maximum value on using 1ml of 0.1% w/v of picric acid.

Stoichiometric relationship

Job's method of continuous variation[18] was employed to determinate the molar ratio between Vildagliptin and picric acid. The concentration of each drug and reagent was adjusted to be 2×10^{-3} M. A series of solutions was prepared in which the total volume of the drug and the reagent was kept at 2.5ml in 10ml calibrated flasks. The solutions were further manipulated as described under the general recommended procedures described above. The absorbance of each solution (against blank treated similarly) was plotted against the drug mole fraction [drug]/[drug+ reagent]. The molar ratio was 1:1 as shown in (Figure 3).
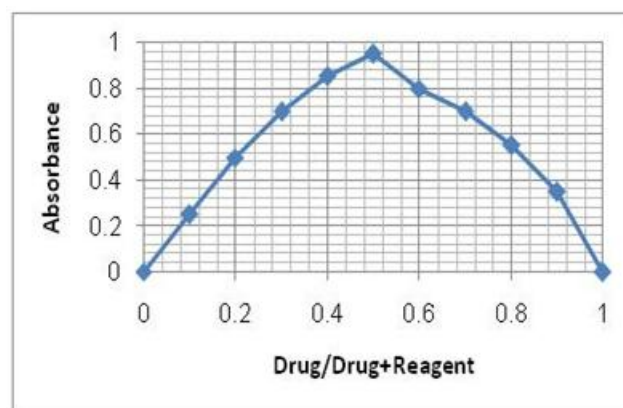
Figure 3: Continuous variation plots of Vildagliptin with reagent

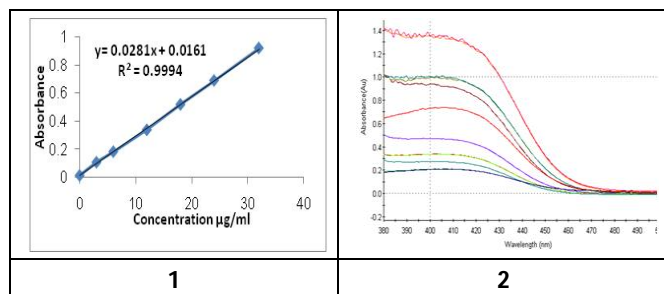### Validation of the proposed method[19]

Linearity

The correlation coefficient (r^2) of the formed product was 0.9994 indicating good linearity (Figure 4) The limit of detection (LOD) and limit of quantification

LOQ) for the proposed method were calculated using the following equations:

LOD = 3.3 σ / S, LOQ = 10 σ / S

Where σ is the standard deviation of intercept. S is the slope of calibration curve. The results are summarized in table 1.

Figure 4: Calibration graph for Vildagliptin after derivatization with picric acid (1) - Vildagliptin – picric acid complex spectra (2).

Table 1: Quantitative parameters of the proposed method

Parameter	Value
Time of reaction (min)	5 min
Stability of color (Hour)	5 hours
Beer's law limits (µg/mL)	3-32
Molar absorptivity (l.mol^{-1} cm^{-1})	9691
Regression equation (y=b+ac)*	Y=0.0281x+0.0161
Correlation coefficient (R^2)	0.9994
Slope, *a*	0.0281
Intercept, *b*	0.0161
Standard deviation of slope	0.000248
Standard deviation of intercept	0.0037
LOD (µg/ml)	0.43
LOQ (µg/ml)	1.32

*$Y = aX + b$, where X is the concentration of Vildagliptin µg ml^{-1}

Table 2: Accuracy and precision of the proposed method for the determination of Vildagliptin

Concentration (µg/ml)	Accuracy* %	Precision			
		Intra- day		Inter –day	
		Recovery ±SD*%	RSD*	Recovery ±SD*%	RSD*
6	99.8%	99.786±1.824	1.828	100.03±1.824	1.823
18	100.05%	100.352±1.48	1.47	99.056±1.657	1.673
32	100.65%	100.67± 0.821	0.815	100.63±1.77	1.76
	Mean±SD*	100.16±0.437			
	RSD*	0.436			

*n=5, SD=standard deviation, RSD=Relative standard deviation

Accuracy and precision

They were checked at three concentration levels, five replicate measurements were recorded at each concentration level. Accuracy was recorded as percent recovery, and by standard addition method. Precision was recorded as relative standard deviation. The calculated relative standard deviations were below 2% indicating good precision of the proposed procedure at both levels of inter-day and intra-day precision; the results are summarized in table 2.

The percent of recoveries of Vildagliptin by standard addition method were in the range of 97.82-99.854 (table 3). This shows the absence of interference from tablet excipients.

Table 3: Results of recovery study by standard-addition method

Amount of drug in formulation (mg)	Amount added (mg)	Recovery ±SD*(%)	RSD*
50	40	98.606±0.585	0.594
50	50	97.82±0.264	0.270
50	60	99.854±0.843	0.845

*n=5

Table 4: Application of the proposed method and reference method for the analysis of dosage form containing Vildagliptin

Method	Brand name	Label claim (mg/tablet)	Amount found	Recovery %* SD±	RSD*
Proposed method	Vildagliptin Biomed	50	48.509	97.018± 0.591 (1.948, 0.17)	0.609
Reference method	-	50	48.23	96.4586±0.2497	0.2589

*n=5, Values in parenthesis are the calculated values of t and F; the tabulated values at 95% confidence limit are 2.78 and 6.39, respectively.

Specificity and effect of excipients

The specificity of the method was investigated by observing any interference encountered from the common tablet excipients, such as starch, lactose anhydrous, microcrystalline cellulose, sodium starch glycolate, magnesium stearate. These excipients did not interfere with the proposed method.

Assay of tablets

The content of tablets was found to be 97.018%±0.591 of the label claim. Statistical analysis (t- and F-tests) showed there was no significant difference between the proposed method and reference method [5]at the 95% confidence level (table 4).

CONCLUSION

A new method which is selective, sensitive, and rapid has been developed and appropriately validated for the assay of Vildagliptin in bulk and pharmaceutical formulations. The analytical reagent is inexpensive and readily available in any analytical laboratory. The method does not require complex procedures (extraction step, heating) or sophisticated equipments and is highly suitable for routine use in quality control laboratories.

REFERENCES

1. EI-BagaryRI, Elkady EF, Ayoub BM, Spectrophotometric methods for the determination of Sitagliptin and Vildagliptin in bulk and dosage forms, Int J Biomed Sci, 7(1), 2011, 55–61.

2. Keating GM, Vildagliptin: A review of its use in type 2 diabetes mellitus, Drugs, 70, 2010, 2089-112.

3. British national formulary, 61, 2011, 6.1.2, 431.

4. Moneeb MS, Spectrophotometric and spectrofluorimetric methods for the determination of saxagliptin and vildagliptin in bulk and pharmaceutical preparations, Bulletin of faculty of pharmacy, Cairo University (bfopcu), 51, 2013, 139-150.

5. Sultana R, Bachar SC, Rahman F, Development and validation of stability indicating assay method of Vildagliptin in bulk and tablet dosage form by RP-HPLC, International journal of pharmacy & life sciences, 4(4), 2013, 2530-2534.

6. Pharne AB, Santhakumari B, Ghemud AS, Jain HK, Kulkarni MJ, A novel bioanalytical method development and validation of Vildagliptin dipeptidyl peptidase IV inhibitor by RP-HPLC method, Int J Pharm PharmSci, 3(4), 2012, 119-123.

7. Gundala U, Bhuvanagiri CS, Nayakanti D, Simultaneous Estimation of Vildagliptin and Metformin in bulk and pharmaceutical formulations by UV Spectrophotometry, Am. J. PharmTech Res, 3(1), 2013, 2249-3387.

8. Mohammad MA, ElKady EF, Fouad MA, Development and validation of a reversed-phase column liquid chromatographic method for simultaneous determination of two novel gliptins intheir binary mixtures with metformin, Eur J Chem, 3, 2012, 152–5.

9. Moneeb MS, LC-UV determination of Vildagliptin either alone or simultaneously with metformin in pharmaceutical preparations, Az J Pharm Sci, 44, 2011, 90–9.

10. Sreelakshmi A, Rao GD, Babu GS, Novel Spectrophotometric Methods for estimation of Naratriptan in pharmaceutical dosage forms, Biosci. Biotech. Res.Asia, 10(2), 2013, 913-916.

11. Bagade SB, Patil SK, Spectrophotometric estimation of Torsemide in tablet dosage form using chemical derivatization technique, International Journal of Pharmaceutical Quality Assurance, 2(1), 2010, 52-55.

12. EI-Mammli MY, Spectrophotometric determination of flucloxacillin in pharmaceutical preparations using some nitrophenols as a complexing agent, Spectrochimica Acta Part A, 59, 2003, 771–776.

13. G Saito, Y Matsunaga, Charge-transfer and proton transfer in the formation of molecular complexes. I. The complex isomerization of some aniliniumpicrates bymelting, Bulletin of the Chemical Society of Japan, 44, 1971, 3328-3335.

14. Abdulrahman AMS, Basavaiah K, Sensitive and Selective Spectrophotometric determination of Gabapentin in Capsules Using Two nitrophenols as Chromogenic Agents, International Journal of Analytical Chemistry, 619310, 2011, 10115.

15. Olajire AA, Chemical derivatization methodologies for UV-Vsible Spectrophotometric determination of pharmaceuticals, Int. J. Pharm. Sci. Rev. Res, 14(2), 2012, 6-24.

16. Singh N, Ahmad A, Spectrophotometric and spectroscopic studies of charge transfer complex of 1-Naphthylamine as an electron donor with picric acid as an electron acceptor in different polar solvents, Journal of Molecular Structure, 977, 2010, 197-202.

17. Prashanth KN, basavaiah K, Simple, sensitive and selective Spectrophotometric methods for the determination of Atenolol in pharmaceuticals through charge transfer complex formation reaction, ActaPoloniaePharmaceutica, Drug Research, 69, 2012, 213-223.

18. Job p, advanced physicochemical experiments, 2nd ed. Edinburgh, oliner and boyd, 1964, 54.

19. ICH harmonized tripartite guideline, validation of analytical procedures: text and methodology, Q2R1, International Conference on Harmonization of Technical requirements for Registration of Pharmaceuticals for Human use, 2005.