DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION AND SPECTROSCOPIC CHARACTERIZATION OF VILDAGLIPTIN USING π -ACCEPTORS IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

Three simple, quick and sensitive methods are described for the spectrophotometric determination of vildagliptin (VLD) in pharmaceutical preparations. The methods are based on formation of colored charge transfer (CT) complexes between VLD as n -electron donor and chloranilic acid (CA), tetrachloro-1,4-benzoquinone (p-chloranil), 7,7,8,8-tetracyanoquinodimethane (TCNQ) as π -acceptors. The colored products were quantitated spectrophotometrically at 520, 535 and 842 nm for CA, p-chloranil and TCNQ methods, respectively. Optimization of different experimental conditions were investigated. Beer's law was obeyed in the concentration range of 20-250 $\mu\text{g mL}^{-1}$, 25-400 $\mu\text{g mL}^{-1}$ and 20-500 $\mu\text{g mL}^{-1}$ for CA, p-chloranil and TCNQ methods, respectively. The formation of the CT-complexes and the sites of interactions were confirmed by elemental analysis using IR, ¹H NMR spectroscopy. Validity of the methods in terms of specificity, linearity, accuracy, precision, robustness, limit of detection and limit of quantitation were evaluated. Good recoveries were obtained for all of the methods indicating that no interference was observed from concomitants usually present in dosage forms. The methods were applied successfully to the determination of VLD in pharmaceutical preparations.

Keywords Charge Transfer Complex, IR spectroscopy, NMR spectroscopy, Pharmaceutical Preparation Spectrophotometry, π -acceptors, Validation, Vildagliptin

INTRODUCTION

Diabetes is a chronic disease that affects the multitude of physiologic pathways known to work together to achieve glycemic homeostasis. It is estimated that approximately 6.6% of the world's population had diabetes in 2010, and this figure is projected to increase to 7-8% by 2030 [1]. Several DPP-4 inhibitors ("gliptins") have been approved by the US Food and Drug Administration (FDA) for the treatment of type 2 diabetes mellitus. These agents have reported efficacy in reducing hemoglobin A1c levels as monotherapy or in combination with other commonly prescribed glucose lowering medications [2]. Vildagliptin (VLD), a member of this class, has been shown good overall safety and tolerability and improving glycaemic control when given as monotherapy or combination therapy [3-4]. VLD improves β - and α -cell sensitivity to glucose by increasing concentrations of active GLP-1 [5]. This results in glucose-sensitive modulation of insulin and glucagon secretion, improving both fasting and postprandial glycaemia, with a low risk of hypoglycaemia. A very low incidence of hypoglycaemia has consistently been reported with VLD across the disease spectrum [6, 7].

Only three HPLC methods were reported for the determination of VLD [8-10]. There is not any spectrophotometric method in literature. Therefore, developing simple, sensitive and precise spectrophotometric methods was aimed. Charge-transfer (CT) reactions have been frequently used spectrophotometrically in the determination of drugs that reacts with some electron acceptors (π -acceptors) such as chloranilic acid (CA), tetrachloro-1,4-benzoquinone (p-chloranil) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) [11-19].

This paper describes the formation of CT complexes between VLD as an electron donor and CA, p-chloranil and TCNQ. The nature and structure of the final products in solid phase have been characterized using ¹H NMR, FTIR spectroscopy.

EXPERIMENTAL

Spectrophotometric measurements were carried out by using a Hitachi spectrometer Model U-2900 equipped with xenon lamp and 1cm quartz cells.

The FTIR spectra of the reactants and the resulting CT complexes were recorded using KBr disc on Thermo Nicolet 6700 FTIR spectrometer. ¹H NMR spectra of the CT complexes, donors and acceptors were recorded in DMSO using Varian Mercury (¹H: 400 MHz) 400 NMR Spectrometer.

Reagents and solutions

VLD was kindly supplied by Megafine (India) and its pharmaceutical

preparation (Galvus®) containing 50 mg of VLD per tablet was obtained from local drug store. All chemicals and reagents were of analytical-reagent grade.

Stock solution of VLD (1 mg mL⁻¹) was prepared in acetonitrile.

TCNQ (Fluka-Neu-Ulm, Germany), CA and p-chloranil Merck (Darmstadt, Germany) were freshly prepared as 0.2%, 0.1% (w/v) solutions in acetonitrile and 1% (w/v) solution in acetone respectively; the solutions were stable for 1 week at 4°C.

General Procedure

Into 5 mL calibrated flasks 0.10-1.25 mL, 0.125-2.0 mL and 0.10-2.5 mL aliquots of VLD stock solution was added for CA, p-chloranil, and TCNQ methods, respectively. The volume was brought to 1.25 mL (for CA), 2.0 mL (for p-chloranil) and 2.5 mL (for TCNQ) with acetonitrile and 0.5 mL of CA, 0.5 mL of p-chloranil, 1 mL TCNQ reagents were added. The reaction mixture was heated for 5 min at 80°C for TCNQ method, and allowed to stand for 5 min at room temperature for both CA and p-chloranil methods. After cooling and diluting to volume 5 mL with acetonitrile, the absorbances were measured at 520, 535 and 842 nm for CA, p-chloranil and TCNQ respectively, against a reagent blank prepared in the same manner. The calibration graph of each method was prepared by plotting absorbance versus concentration of VLD.

Synthesis of the solid CT complexes

The solid CT complexes were synthesised by adding a saturated solution of VLD (303.4 mg, 1x10⁻³ mol) in acetonitrile to each reagent solution (to a saturated solution of CA (313.5 mg, 1.5x10⁻³mol), p-chloranil (328 mg, 1.3x10⁻³mol), TCNQ (204 mg, 1x10⁻³mol). The mixtures were stirred for about 30 min and then filtered off to avoid the contamination with any unreacted species. The solutions were then left nearly 2 h to obtain the solid CT complexes, filtered and washed several times with minimum amounts of acetonitrile in order to remove the residual reagents and drug substance.

Assay procedure for tablets

Ten tablets were transferred to a 250 mL volumetric flask. About 125 mL of acetonitrile were added and then extraction was performed mechanically for 20 minutes and sonicated for 20 more minutes. The volume was brought to 250 mL with acetonitrile and the final solution was filtered. Aliquots of the filtrate was diluted further with acetonitrile and then preceded as described under preparation of the calibration curve. The nominal contents of the tablets were calculated using either the calibration graph or the corresponding regression equation.

RESULTS AND DISCUSSION

As a product of the interaction between VLD and CA, p-chloranil, TCNQ; CT complexes were occurred which absorb light at 520, 535 and 842 nm, respectively (Fig. 1).

Fig.1 (a) Absorption spectrum of $150 \mu\text{g mL}^{-1}$ VLD-CA complex against reagent blank, (b) Absorption spectrum of $150 \mu\text{g mL}^{-1}$ VLD-p-chloranil complex reagent against reagent blank and (c) Absorption spectrum of $250 \mu\text{g mL}^{-1}$ VLD-TCNQ complex reagent against reagent blank.

The CT reactions are shown in Figure 2

Fig.2 The reactions between VLD and π -acceptors in acetonitrile.

The effect of different parameters on the complex formation was studied to determine optimum conditions for all of the assay procedures.

Optimization procedure

Choice of solvent

Different solvents included acetonitrile, chloroform, methanol, acetone, ethanol, 1,4-dioxane, and methylene chloride were investigated in order to select the suitable solvent for CT formation medium. Acetonitrile (for chloranilic acid and TCNQ) and acetonitrile: acetone mixture (1:1) (for p-chloranil) were considered to be ideal solvents for the most effective complex formation reaction.

Reagent concentration

The effect of reagent (π -acceptor) concentration (by volume) on the reactions were investigated. Different volumes of TCNQ (0.2% (w/v)), CA (0.1% (w/v)) and p-chloranil (1% (w/v)) were added to a fixed concentration of VLD. 0.5 mL of CA and p-chloranil and 1 mL TCNQ solution was found to be sufficient for quantitative determination of VLD (Fig. 3).

Fig.3 Effect of volumes of CA (0.2%, w/v) and p-chloranil (0.2%, w/v) and TCNQ (0.2%, w/v) on the development of the reaction product of VLD with (▲) CA, (■) p-chloranil and (♦) TCNQ.

Reaction time

The optimum reaction time for all of the methods was determined by investigating the color development at room temperature and 60-80°C. Complete color development was attained after 5 min for TCNQ at 80°C, whereas for p-chloranil and CA, room temperature for 5 min. was optimum (Fig 4). The colors remained stable for 12 h for all complexes.

Fig.4 The effect of temperature and time of heating on the reaction of TCNQ (♦, at 80°C), CA (▲, at room temperature) and p-chloranil (■, at room temperature) reagents with VLD.

Stoichiometry of the reaction

The molar ratio of the reagent in reaction mixture was studied for all methods according to Job's method of continuous variation [20]. Utilizing equimolar solutions of VLD and the reagents provided the most effective reactions (the reaction stoichiometry was found to be a good approximation 1:1 ratio (drug/reagent) for all methods).

Method validation

The developed analytical methods were validated in accordance with the ICH guideline Q2 (R1) [21]. Under the described experimental conditions, calibration graphs were constructed for all of the methods and Table 1 summarizes the values for Beer's law limits, regression equation, correlation coefficients, molar absorptivity, Sandell's sensitivity, limit of detection (LOD) and limit of quantification (LOQ) for each method. As can be seen from the Table 1, linear relationship for all methods was found between the absorbance at λ_{max} and the concentration of the drug in the ranges of 20-250 $\mu\text{g mL}^{-1}$, 25-400 $\mu\text{g mL}^{-1}$ and 20-500 $\mu\text{g mL}^{-1}$ for CA, p-chloranil and TCNQ methods, respectively. The CA method was the most sensitive one, with a high ϵ value. LOD and LOQ were determined using the formula: $\text{LOD or LOQ} = \kappa \text{SDa/b}$, where $\kappa = 3$ for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The inter- and intra-day precisions were examined by analysis of VLD with concentrations of 20, 100 and 250 $\mu\text{g mL}^{-1}$ for CA method, 25, 100 and 400 $\mu\text{g mL}^{-1}$ for p-chloranil method and 20, 100 and 500 $\mu\text{g mL}^{-1}$ for TCNQ method (each $n=5$) for seven consecutive days. The % RSD values for intra-day precision %, and inter-day precision were found for all developed methods, which indicate good repeatability. The obtained results are summarized in Table 1.

Table 1 Results of validation parameters for proposed methods.

	Using CA reagent	Using p-chloranil reagent	Using TCNQ reagent
Linearity range ^a (µg mL ⁻¹)	20-250	25-400	20-500
Molar absorptivity (l mol ⁻¹ cm ⁻¹)	3.55 x 10 ³	2.03 x 10 ³	1.8 x 10 ³
Sandel's sensitivity (µgcm ⁻² per 0.001 absorbance unit)	0.85	0.13	0.17
<i>Precision</i>			
Intra-day ^b , RSD %	0,95	0,87	0.68
Inter-day ^c , RSD %	1.12	1.28	1.39
<i>Regression equation</i> ^d			
Slope ± SD	0.0034 ± 0.00005	0.0021 ± 0.00001	0.0016 ± 0.00003
Intercept ± SD	0.0187 ± 0.00013	0.0755 ± 0.00019	0.0981 ± 0.00023
Correlation coefficient, <i>r</i>	0.9995	0.9997	0.9994
LOD (µg mL ⁻¹)	0.12	0.28	0.43
LOQ (µg mL ⁻¹)	0.38	0.92	1.44

To check accuracy of the proposed methods, standard addition technique was applied. A different amount of pure sample solution was added to three different concentrations of standard drug solutions and assayed. The percent recovery of the added standard to the assay samples was calculated from:

$$\text{Recovery \%} = [(C_t - C_u) / C_a] \times 100$$

where C_t is the total concentration of the analyte found, C_u is the concentration of the analyte present in the formulation, and C_a is the concentration of the pure analyte added to the formulation. The results of analysis of the commercial dosage forms and the recovery study were shown in Table 2. The average percent recoveries obtained were quantitative (101.11%, 101.46 % and 101.33 % for CA, p-chloranil and TCNQ methods, respectively), indicating good accuracy of the methods.

Table 2 Results of recovery studies by standard addition method.

Method	Amount taken (µg mL ⁻¹) ^a	Amount added (µg mL ⁻¹)	Total amount found ^b (µg mL ⁻¹) (Mean± S.D. ^c)	Recovery (%)	RSD (%)
Using CA reagent	100	20	120.32±1.14	101.62	0.95
		100	200.147±1.58	100.15	0.79
		150	252.360±2.58	101.57	1.02
Using p-chloranil reagent	100	25	125.47±1.34	101.88	1.07
		100	201.66±1.87	101.66	0.93
		300	402.54±2.56	100.85	10.64
Using TCNQ reagent		20	120.36±1.14	101.80	0.95
	100	200	302.54±1.58	101.27	0.79
		400	503.65±3.87	100.91	0.77

The applicability of the proposed methods was tested by the determination of VLD in tablets. The obtained results are satisfactorily accurate and precise as indicated by the excellent % recovery and SD<2 (Table 3). Experiments showed that there was no interference from the additives and excipients, e.g. lactose, glucose, fructose, magnesium stearate and starch.

Table 3 Analysis of VLD in tablet containing 50 mg of the drug (n=5) 3.3 IR spectra of the CT acceptor complexes.

	Using CA reagent (Mean± S.D. ^c)	Using p-chloranil reagent (Mean± S.D. ^c)	Using TCNQ reagent (Mean± S.D. ^c)
Mean ± S.D	50.45 ± 0.31	50.31 ± 0.12	50.34 ± 0.09
Recovery (%)	100.90	100.62	100.68
RSD (%)	0.61	0.23	0.17

^aGalvus tablet (50 mg)^bFive independent analyses.^cStandard deviation

IR spectra of the CT Complexes VLD showed the expected -NH and -OH stretch at 3420 cm⁻¹ as a broad peak of -OH stretching overlapped with the sharp stretching band of -NH group. Together with this the weak stretching band of -C≡N at 2239 cm⁻¹ and the amide like ketone stretching at 1650 cm⁻¹ were observed. The C-O stretch at 1359 confirms the presence of the overlapped -OH group. The IR spectra of CA gives an -OH peak at 3233 cm⁻¹, C=O stretch at 1665 and 1626 cm⁻¹ and C-O 1359 cm⁻¹ which coincides with previously reported data [21]. In the IR spectra of VLD-CA, the complete disappearance of -NH peak at 3420 cm⁻¹ is observed while the broadened peak of -OH is still presented. On the other hand the -OH peak of CA at 3233 cm⁻¹ is being recorded together with that of VLD. Previously the presence of -NH₃⁺ has been reported by the observation of the multiple peaks at the 2500-3000 cm⁻¹ [22] which is not seen in our spectra. All of the above mentioned evidences show the formation of a chemical bound between VLD and CA. The IR spectra of TCNQ reported a -C≡N stretch at 2223 cm⁻¹ [22] and C=C stretching at 1619 cm⁻¹. The chemical bound made between VLD and TCNQ is being exhibited on the IR spectra by the shifting of the nitrile group to 2253 cm⁻¹ and disappearance of -NH peak at 3420 cm⁻¹. The IR spectra of p-chloranil gives C=O stretch at 1688 and 1677 cm⁻¹ and C-O 1315 cm⁻¹. In the IR spectra of VLD- p-chloranil, again the -NH peak at 3420 cm⁻¹ is being lost and a new C-N stretch is being observed at 1103 cm⁻¹ indicating the foration of a new C-N bound between VLD and p-chloranil molecules.

¹H-NMR spectra of the CT Complexes

Our results obtained from the NMR study of VLD is in well agreement with the previously published data [23, 24]. The most characteristic peaks of the NMR spectrum are the peaks at 4.70 (0.8 H, q) and 5.17 (0.2 H, d) ppm related to the isomers of the α proton of C-5, a member of ring A. The structure of VLD can be seen in Figure 2. The %20 occurrence of this proton in β position causes the appearance of this proton in two different peaks in NMR spectrum. The second important point in the spectrum is the presence of all protons of the ring system B in only two broadened singlet peaks at 1.40 ppm for 8 H (likely a singlet for 6H and a singlet for 2H under it, exposed around 1.39 ppm) and at 1.47 ppm for 6 H. Although the first impression of the ring system B is 14 protons with different chemical environments, rotating the 3D structure of the structure around amine group shows the equal distances of all protons from the hydroxyl and amine groups at two sides of multi-cyclic system (Fig. 5) causing the occurrence of two broadened singlet peaks in the ¹H-NMR spectrum. The NH-CH₂ protons are observed at 3.31 ppm (2H, d), while the protons of etilene group neighbour to the nitrogen of pyrrolidine ring is observed at δ 3.41 (¹H, dd) and δ 3.58 (¹H, m). A broadened singlet at δ 4.39 has been attributed to NH group.

Complexation of VLD with TCNQ, CA and p-chloranil caused the complete disappearance of the NH peak at δ 4.39 which shows that all reagents make chemical bound with this group. Together with that, the above mentioned broadened singlet peak of eight protons at δ 1.40 splits to two multiplet peaks at 1.42 and 1.54 ppm corresponding to two and six protons respectively. Probably the attached reagent to the amine group causes a significant change in the chemical environment of the ring system B. Moreover the protons of CH₂-NH show a shift to down field from 3.31 ppm to 3.7 ppm due to the connection of electrophilic groups to the neighboring NH group in the all cases. On the other hand, the presence of complexation agents prevents the formation of conformational isomers of the ring A which consequently decreases the occurrence of the proton belong to C-5 in β position. This fact results in almost disappearance of the peak at 5.17 ppm. In case of complexation of VLD with TCNQ and p- chloranil, a new peak corresponding to one proton around 4

ppm demonstrates the reduction of the agent at the site of connection. Thus the suggested results of reaction between VLD and CA, p-chloranil and TCNQ is as shown in Figure 2. Since there is no significant difference between NMR spectra of VLD complexes, the spectrum of VLD-p-chloranil is given as example (Fig 5 and 6).

Fig.5 Chemical structure of ring system B **Fig.6** 1H-NMR spectrum of VLD **Fig.7** 1H-NMR spectrum of VLD- p-chloranil complex

CONCLUSION

Three selective, simple and less time consuming spectrophotometric methods using for CA, p-chloranil and TCNQ reagents were described for determination of anticonvulsant drug substance VLD in this study. CA method was superior as compared with the other two methods according to higher molar

absorptivity and lower detection limits. The described methods are suitable for the determination of VLD in pharmaceutical formulations without interference from excipients and can be easily applied to quality control laboratories for the routine analysis of VLD in raw materials and pharmaceutical formulations.

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