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New Analytical Methods for the Determination of Two Gliptin Drugs In Pharmaceutical Formulations and Urine Samples

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ABSTRACT

New ion – associate complexes of the two gliptin drugs, vildagliptin and saxagliptin were precipitated with tetraiodometrcurate and ammonium reineckate and the excess unreacted metal complex was determined. New methods were given for the determination of these drugs in pure solutions, pharmaceutical formulations and urine samples using atomic absorption and atomic emission spectrometry. The drugs can be determined by the afford methods in the ranges 0.68 – 74.69 and 0.70 – 77.42 $\mu\text{g} / \text{ml}$ for vildagliptin and saxagliptin, respectively.

Keywords: Ion-associate complexes, atomic absorption, atomic emission, , pharmaceutical analysis.

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INTRODUCTION

Since the discovery of gliptins, a dipeptidyl peptidase-4 (DDP-4) inhibitors, a number of structural modifications have been made in order to increase their antidiabetic activity. Studies have shown that DDP-4 inhibitors (Vildagliptin, and Saxagliptin) showed better glycemic control. Vildagliptin HCl;(Vd)[(S)-1-[N-(3-hydroxy-1-adamantyl) glycy]pyrroindine-2-carbonitrile hydrochloride] is a potent oral hypoglycemic agent. Saxagliptin HCl; (Sx) is chemically named as (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabi-cyclo[3.1.0]hexane-3-carbo-nitrile hydrochloride.¹

Vildagliptin is an example of this new class of antidiabetic drugs, acts by inhibiting DPP-4 enzyme, which helps in maintaining the high level of incretin hormones (GLP-1 and GIP). GLP-1 and GIP maintain normal blood glucose level by different mechanisms, like stimulating the islets of pancreatic gland to release insulin, by suppressing the secretion of glucagon, by reducing the gastric clearance and by decreasing the food intake. In addition, there is a less chance of hypoglycemia and gain in the body weight.²⁻⁵ Development of quantitative analytical method for the estimation of vildagliptin is very important. Different analytical methods have been described for the quantification of vildagliptin from formulations and biological samples, including spectrophotometric methods⁶⁻⁷, HPLC⁸⁻¹², GC-MS¹³ and capillary electrophoresis.^{14, 20 - 21} Recently, few LC-MS/MS methods were described for the estimation of vildagliptin from plasma.¹⁵⁻²⁰ Literature survey represented that saxagliptin can be determined by several methods including; spectrophotometry¹⁷⁻²⁰, TLC²¹⁻²³ and HPLC methods.²⁴⁻⁴² Many of these methods involve several time-consuming manipulations, extraction steps, derivatization reactions that are liable to various interferences, and are not applicable to colored and turbid solutions.

Vildagliptin and saxagliptin are very important pharmaceutical compounds. Therefore, we found it important to prepare new ion-associates containing these drugs and to study and elucidate their chemical structures. Also the work present a new rapid method for the determination of these drugs after transformation into the ion-associates.

The chemical structure of vildagliptin and saxagliptin drugs are shown in Figures (1 and 2). The use of simpler, faster, less expensive and sensitive method is desirable.

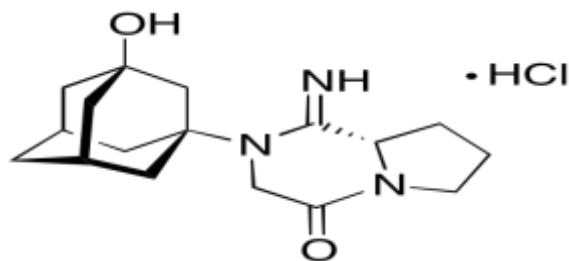


Figure 1: Chemical structure of Vildagliptin Hydrochloride

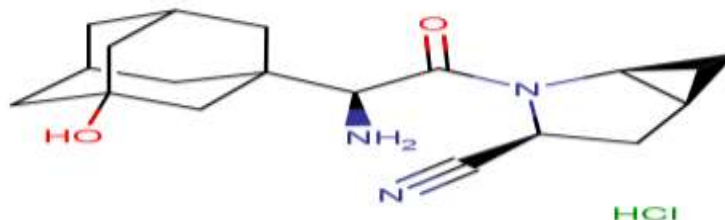


Figure 2: Chemical structure of Saxagliptin Hydrochloride

Although, Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) are rapid methods and have a very low detection limits which can not be reached by most of other methods. The present study includes new AAS and ICP-AES methods for the determination of the investigated drugs. The method are based on precipitating the ion-associates formed as a result of the combination of these drugs with an excess of $[\text{HgI}_4]^{-2}$ and $[\text{Cr}(\text{NH}_3)_2(\text{SCN})_4]^{-1}$. The equilibrium concentration of the metal ion present as the soluble inorganic complex ion in the supernatant solution was determined using atomic absorption and emission.

Analytical grade reagents and doubly-distilled water were used in the preparation of all solutions.

Vildagliptin HCl was a gift sample from Novartis, Egypt. Saxagliptin HCl was kindly supplied by Bristol-Myers Squibb/AstraZeneca (United Kingdom). The pharmaceutical preparations of vildagliptin (Galvus 50 mg / tablet) and for saxagliptin (Onglyza and Kombiglyze XR 5 mg / tablet) were obtained from local market. Ammonium reineckate was obtained from Aldrich.

APPARATUS

Atomic absorption measurements were made on AA-6650 Shimadzu atomic absorption spectrophotometer. Inductively coupled plasma atomic emission measurements were carried out using ICPE- 9000 Shimadzu plasma atomic emission spectrometer. The pH of the solutions was measured using an Orion Research Model 701A digital pH-meter and Conductimetric measurements were carried out using conductivity measuring bridge type M.C.3 model EBB/10

($K_{\text{cell}} = 1$); [Chertsey, Surry, England]. The IR absorption spectra were obtained by applying the KBr disk technique using a Pye Unicam SP – 300 infrared spectrometer.

PREPARATION OF THE STANDARD SOLUTIONS

Standard solution of chromium was prepared by weighing 1.0 g of a high-purity sample (chromium shot), transferring it to a 1-liter measuring flask and then adding 50 ml of concentrated HNO_3 . After complete dissolution, the solution was filled to the mark with distilled water. The 1000 $\mu\text{g}/\text{ml}$ solution was stored in plastic bottles which had been presoaked in dilute HNO_3 . The solution was stable for approximately one year. Mercury (II) iodide, potassium iodide and mercury atomic absorption standard solution 1000 mg/ml of Hg in 10% HNO_3 (Aldrich).

EMISSION AND ABSORPTION MEASUREMENTS

Analytical Parameters for the Measurement of Cr using ICP-AES are listed in Table 1. Mercury was measured by AAS using a hollow cathode lamp of Hg, under the following conditions; wavelength 253.7 nm, slit width 0.7 nm, relative noise 4.2, detection limit 0.28 mg/ml, linear dynamic range, 0.01–100, lamp current 5 mA, and integration time 3 s, the flame used was the acetylene-air mixture. The instruments were equally adequate for present purposes and were used according to availability. The atomic spectrometry was calibrated as in the previously reported work.⁴³⁻⁴⁵

Table 1: Analytical Parameters for the Emission Measurement of Cr Using ICP-AES

Element	Wavelength (nm)	Order	Plasma position	DL (mg/L)	LDR (mg/L)	BEC (mg)	RSD x BEC
Cr	267.71	84	0	0.01	0.1-1000	0.4	7 x 0.7

Note. DL, detection limit; LDR, linear dynamic range; BEC, background equivalent concentration; RSD, relative standard deviation. For all elements: state, ion; entrance slits, 50 x 300 μm ; exit slits, 100 x 300 μm .

Determination of Solubility Of The Ion – Associates

The solid ion-associate was added in excess to a solution of the optimum pH and ionic strength. The solution was shaken for 4-6 hs and left to stand for a weak to attain equilibrium. Then the saturated solution was filtered into a dry beaker (rejecting the first few ml of filtrate). The equilibrium concentration of the metal ion present in the form of a soluble inorganic complex was measured using atomic spectrometry. Hence, the solubility (S) of the precipitate was evaluated, from which the solubility product of the ion-associate was calculated.

Conductometric Measurements

The stoichiometry of the ion-associates was elucidated also by conductometric titrations⁴⁶ of the drugs with $[\text{HgI}_4]^{-2}$ and $[\text{Cr}(\text{NH}_3)_2(\text{SCN})_4]^{-1}$ solutions.

Analytical Determination Of Vildagliptin And Saxagliptin In Aqueous Solutions

Aliquots (0.05 - 5.5 mL) of 0.001 mol L⁻¹ drug solutions were quantitatively transferred to 25 mL volumetric flasks. To each flask 1.0 mL of 0.01 mol L⁻¹ standard solution of [HgI₄]⁻² and [Cr(NH₃)₂(SCN)₄]⁻¹ was added and the volume was completed to the mark with the aqueous solutions of the optimum pH and ionic strength (prepared from HCl and NaOH). The solutions were shaken well and left to stand for 15 min then filtered through Whatman P/S paper (12.5 cm). The equilibrium metal ion concentration in the filtrate was determined using AAS or ICP-AES. The consumed metal ion (Hg or Cr) in the formation of ion-associates was calculated, and the drug concentration was determined indirectly.

Analytical Determination of Vildagliptin And Saxagliptin In Pharmaceutical Preparations and Urine Samples

The vildagliptin - containing pharmaceutical preparations (Galvus 50 mg tablets) and for saxagliptin (Onglyza and Kombiglyze XR 5 mg tablets) were successfully assayed using the present method. Sampling were made by grinding (20 tablets of each), then taking 0.75 - 65.25, 5.25 - 60.25 and 8.25 - 68.45 µg / ml of Galvus, Onglyza and Kombiglyze XR tablets, respectively. Urine samples were obtained from 20 patients after 4 – 8 hours of taking dose. In all cases the tablets and urine samples were analyzed at the optimum condition solution applying the above described procedure.

RESULTS AND DISCUSSION

The results of elemental analysis (Table 2) of the produced solid ion associates reveal that two drug cations form ion associates with one [HgI₄]⁻² and only one drug cation combines with [Cr(NH₃)₂(SCN)₄]⁻¹ to form a 1:1 ion associate. These results are comparable to the previously reported results.⁴⁷⁻⁴⁹

Conductometric titrations of the investigated inorganic complexes with Vd and Sx were performed to give insight into the stoichiometric compositions of the ion- associates formed in solutions. In case of ion associates with [HgI₄]⁻², the characteristic curves break at a molecular ratio ([drug] / [x]ⁿ⁻) of about 2, confirming the formation of 2:1 (drug : x²⁻) ion associates but in the case of the reineckate anion where the curve exhibits a sharp break at the 1:1 molecular ratio. The results obtained coincide with the elemental analysis of the precipitated ion- associates.

Table 2: Elemental analysis, composition and some physical properties of vildagliptin and saxagliptin ion – associates

Ion-associate composition	m. p. 0c	Molar ratio	Color	% Found (calculated)			Metal (Hg or Cr)
				C	H	N	
(C17 H25 N3O2)2 [HgI4	297	2:1	white	31.08 (31.04)	3.84 (3.80)	6.42 (6.39P)	15.29 (15.25)
(C17 H25 N3O2) [Cr (NH3)2 (SCN)4]	386	1:1	pink	40.62 (40.58)	5.03 (4.99)	20.34 (20.29)	8.42 (8.37)
(C18 H25 N3O2)2 [HgI4]	265	2:1	White	32.34 (32.27)	3.78 (3.74)	6.33 (6.28)	15.03 (14.98)
(C18 H25 N3O2) [Cr (NH3)2 (SCN)4]	394	1:1	pink	41.83 (41.77)	4.97 (4.90)	19.96 (19.90)	8.27 (8.21)

The optimum pH and ionic strength values (Table 3) have been elucidated by determining the solubility of the ion-associates in HCl-NaOH solutions of different pH values and ionic strengths. The best were those exhibiting lowest solubility values.

Analytical Determination of Vildagliptin And Saxagliptin In Aqueous Solutions, Pharmaceutical Preparations And Urine Samples

Vildagliptin and saxagliptin were determined precisely and accurately in aqueous solutions at their optimum conditions of pH and ionic strength (Table 4), in pharmaceutical preparations and urine samples using the present method. The results given in Table 4 reveal that recoveries were in the range 98.93 - 100.08 %, reflecting the high accuracy in addition to the high precision indicated by the very low values of the relative standard deviation.

Generally, the present method is as good as those reported before where, 0.68 – 74.69 and 0.70 – 77.42 $\mu\text{g} / \text{ml}$ solutions of vildagliptin and saxagliptin using $[\text{HgI}_4]^{-2}$ and $[\text{Cr}(\text{NH}_3)_2(\text{SCN})_4]^{-1}$ were determined, respectively, which means that this method is applicable over a wider concentration range than that of the previously published spectrophotometric methods⁶⁻⁷ in which vildagliptin was determined in the ranges 8 – 32 and 30 – 70 $\mu\text{g} / \text{ml}$, respectively and also than that of the spectrophotometric method²⁰ and RP-HPLC method⁴² in which saxagliptin was determined in the ranges 5 – 40 and 10 – 50 $\mu\text{g} / \text{ml}$, respectively.

In pharmaceutical analysis it is important to test the selectivity toward the excipients and the fillers added to the pharmaceutical preparations. Fortunately, such materials mostly do not interfere. It is clear from the results obtained for the pharmaceutical preparations (Table 4) that these excipients do not interfere.

In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression [50] of observed drug concentration against the theoretical values (five points) was calculated. The student`s t-test⁵⁰ (at 95% confidence level) was applied to the slope of the regression line which showed that it did not differ significantly from the ideal value of unity. Hence, it can be concluded that there are no systematic differences between the determination and the true concentration over a wide range. The standard deviations (SD) can be considered satisfactory at least for the level of concentrations examined.

Table 3: Solubility and solubility product of vildagliptin,(Vd) and saxagliptin (Sx) ion-associates at their optimum conditions of pH and ionic strength (μ) values at 25° C

Drug	Ion – associate	pH	μ	ps	pksp
Vd	(C17 H25 N3O2) 2 [HgI4	5.0	0.6	7.34	6.62
Vd	(C17 H25 N3O2) [Cr (NH3)2(SCN)4]	4.0	0.5	10.36	20.72
Sx	(C18 H25 N3O2) 2 [HgI4]	5.0	0.7	7.05	15.75
Sx	(C18 H25 N3O2) [Cr (NH3)2 (SCN)4]	4.0	0.4	9.65	19.30

p^s : -log solubility

pk_{sp} : -log solubility product

Table 4: Determination of vildagliptin, (Vd) and saxagliptin, (Sx) in aqueous solutions, pharmaceutical preparations and urine samples by AAS and ICP-AES

Sample	Amount taken (μ g)	Mean recovery (%)	Mean RSD (%)
Using [HgI₄]⁻² *			
Pure Vd solution	0.68 – 74.69	99.95	0.6
Galvus tablets ^a (50 mg Vd / tablet)	0.75 - 65.25	99.94	0.7
Urine after 4 hs	20.75 - 55.25	99.92	0.6
Urine after 8 hs	5.25 - 25.25	99.93	0.5
Using [HgI₄]⁻² *			
Pure Sx solution	0.70 – 77.42	99.97	0.8
Onglyza tablets ^b (5 mg Sx / tablet)	5.25 - 60.25	99.95	0.7
Kombiglyze XR ^b (5 mg Sx / tablet)	8.25 - 68.45	99.95	0.6
Urine after 4 hs	25.50 - 45.25	99.96	0.7
Urine after 8 hs	5.25 - 25.25	99.97	0.6
Using [Cr (NH₃)₂ (SCN)₄]^{-1**}			
Pure Vd solution	0.68 – 74.69	100.08	0.7
Galvus tablets ^a (50 mg Vd / tablet)	0.75 - 65.25	100.06	0.5
Urine after 4 hs	20.75 - 55.25	100.05	0.6
Urine after 8 hs	5.25 - 25.25	100.06	0.5
Using [Cr (NH₃)₂ (SCN)₄]^{-1**}			
Pure Sx solution	0.70 – 77.42	98.96	0.6
Onglyza tablets ^b (5 mg Sx / tablet)	5.25 - 60.25	98.97	0.7
Kombiglyze XR ^b (5 mg Sx / tablet)	8.25 - 68.45	98.93	0.6

Urine after 4 hs	25.50 - 45.25	98.94	0.7
Urine after 8 hs	5.25 - 25.25	98.96	0.5

RSD : Relative Standard Deviation (five determinations)

* By AAS ** By ICP-AES

^a Novartis Pharmaceuticals, Uk, Ltd.

b Astrazeneca Pharmaceuticals Lp.

CONCLUSION

The present method is as good as those reported before where, 0.68 – 74.69 and 0.70 – 77.42 µg / ml solutions of vildagliptin and saxagliptin using $[HgI_4]^{-2}$ and $[Cr(NH_3)_2(SCN)_4]^{-1}$ were determined, respectively, which means that this method is applicable over a wider concentration range than that of the previously published spectrophotometric methods ⁶⁻⁷ in which vildagliptin was determined in the ranges 8 – 32 and 30 – 70 µg / ml, respectively and also than that of the spectrophotometric method²⁰ and RP-HPLC method ⁴² in which saxagliptin was determined in the ranges 5 – 40 and 10 – 50 µg / ml , respectively. Although the present method is more time consuming than some other methods, it exhibits fair sensitivity and accuracy. Moreover, the reproducibility of the results is superior to those obtained with other methods.

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