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Compatible validated spectrofluorimetric and spectrophotometric methods for determination of vildagliptin and saxagliptin by factorial design experiments ☆

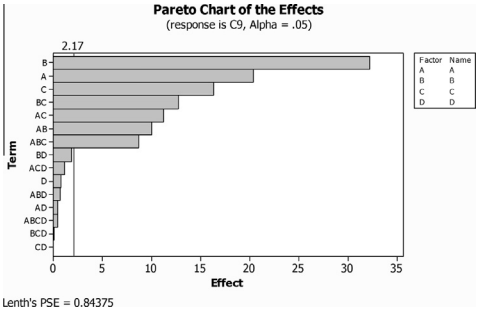
Omar Abdel-Aziz, Miriam F. Ayad, Mariam M. Tadros *

Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt

HIGHLIGHTS

- The first method is based on dansylation of vildagliptin.
- The spectrophotometric method is based on the charge transfer of saxagliptin.
- The other spectrophotometric method is based on Hantzsch reaction of saxagliptin.
- The variables were studied using experimental factorial design.
- The developed methods were validated for quality control of vildagliptin and saxagliptin.

GRAPHICAL ABSTRACT



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ABSTRACT

Simple, selective and reproducible spectrofluorimetric and spectrophotometric methods have been developed for the determination of vildagliptin and saxagliptin in bulk and their pharmaceutical dosage forms. The first proposed spectrofluorimetric method is based on the dansylation reaction of the amino group of vildagliptin with dansyl chloride to form a highly fluorescent product. The formed product was measured spectrofluorimetrically at 455 nm after excitation at 345 nm. Beer's law was obeyed in a concentration range of 100–600 μg ml^{-1}. The second proposed spectrophotometric method is based on the charge transfer complex of saxagliptin with tetrachloro-1,4-benzoquinone (*p*-chloranil). The formed charge transfer complex was measured spectrophotometrically at 530 nm. Beer's law was obeyed in a concentration range of 100–850 μg ml^{-1}. The third proposed spectrophotometric method is based on the condensation reaction of the primary amino group of saxagliptin with formaldehyde and acetyl acetone to form a yellow colored product known as Hantzsch reaction, measured at 342.5 nm. Beer's law was obeyed in a concentration range of 50–300 μg ml^{-1}. All the variables were studied to optimize the reactions' conditions using factorial design. The developed methods were validated and proved to be specific and accurate for quality control of vildagliptin and saxagliptin in their pharmaceutical dosage forms.

Introduction

Vildagliptin (VLG), *S*-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile (Fig. 1A) and saxagliptin (SXG), (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile (Fig. 1B) are novel oral hypoglycemic

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* Corresponding author. Tel.: +20 1223345260; fax: +20 224051107.
E-mail address: mariam.tadros@hotmail.com (M.M. Tadros).

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drugs of the dipeptidyl peptidase 4-inhibitor class (DPP-4) [1,2]. DPP-4 inhibitors represent a new therapeutic approach for treatment of type-II diabetes; that functions to stimulate glucose-dependent insulin release and reduce glucagon's levels. This is done through inhibition of the inactivation of incretins, particularly glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), thereby improving glycemic control [3–5].

Literature review showed many methods for determination of VLG; based on the charge transfer complexes of VLG with 2, 3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 7,7,8,8-tetracyanoquinodimethane (TCNQ) and tetrachloro-1,4-benzoquinone (*p*-chloranil) [6]. Also; literature survey reveals that many chromatographic methods have been developed [7–13]. In addition; SXG has been estimated by LC–MS/MS [14], HPLC methods [15–18,9] and by spectrophotometric method, in which SXG was estimated at 208 nm in methanol [19]. Another spectrophotometric method based on charge transfer reaction using DDQ and TCNQ was reported [20].

The aim of the first method is to present new spectrofluorimetric method based on the dansylation reaction for determination of VLG in bulk and in pharmaceutical dosage form. Furthermore, the established method should be rapid to be applied for routine quality control analysis of VLG in pharmaceutical dosage form.

Spectrofluorimetry has long been applied in the field of pharmaceutical analysis of many drugs [21–23]. A necessary condition for a compound to fluoresce is that it absorbs light in the UV or visible region of the spectrum or reacts with a reagent to give a fluorescent product. Accordingly, a certain group in the compound as the primary amine for example react with the reagent to obtain a highly fluorescent product [21–23]. Dansyl chloride is a useful derivatizing agent for primary amines, secondary amines, imidazoles and phenols. Several pharmaceutical compounds have been determined through this approach [24–30].

The aim of the second and third methods is to present new spectrophotometric methods based on a charge transfer reaction and Hantzsch reaction for the determination of SXG in bulk and pharmaceutical dosage forms.

Spectrophotometry continues to be very popular, because of its simplicity and versatility. Charge transfer reactions have been widely used for determination of electron donating compounds through interaction with π-acceptors [31–41]. Among the electron acceptors mostly used in literature is tetrachloro-1,4-benzoquinone (*p*-chloranil) [39–41].

Hantzsch reaction of primary amines with formaldehyde and acetyl acetone forms a yellow colored product that can be measured spectrophotometrically. The quantification of many drugs in its bulk and pharmaceutical dosage forms were carried out using Hantzsch reaction [42–48].

Hantzsch reaction is a known condensation reaction that was reported in the literatures as a useful pathway for pyrrole and pyridine synthesis, In the same manner, acetylacetone together with formaldehyde react with aliphatic amines by Hantzsch reaction forming a yellow product that can be measured spectrophotometrically [42–48]. The proposed method for determination of SXG (primary amine compound) was based on Hantzsch condensation reaction using acetylacetone as β-diketone and formaldehyde as an aldehyde to form a colored condensation product.

Optimization of the reaction conditions of all the proposed methods were optimized by experimental design using Minitab® program with the advantage of finding a classic tool for estimating the mutual significance of multiple factors and fulfill most of the important optimality criteria [49–51].

Experimental

Instrumentation

BIO-TEK spectrofluorimeter, Italy, SFM 25 software was used for spectrofluorimetric measurements while the spectrophotometric determinations were done using a Double-beam Shimadzu (Japan) 1601 PC UV–Visible spectrophotometer connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu). Jenway digital pH meter was used to adjust and determine the hydrogen ion concentration (pH) of the buffer solutions.

Samples

Pharmaceutical grade VLG, certified to contain 99.70% and Galvus® tablets nominally containing 50 mg VLG per tablet (batch No. V6498) were kindly supplied from Novartis Europharm limited company (London, U.K.). Pharmaceutical grade SXG certified to contain 99.85% and Onglyza® tablets (batch No. 0J57932) nominally containing 5 mg of SXG per tablet were kindly supplied by Bristol-Myers Squibb/AstraZeneca EEIG (United Kingdom).

Reagents

1-Dimethyl aminonaphthalene-5-sulphonyl chloride (dansyl chloride), purchased from Sigma (St. Louis, USA). A stock solution containing 0.1% of dansyl chloride was freshly prepared in acetone and was further diluted with the same solvent to obtain 0.001% solution (solution a) [24]. Carbonate:bicarbonate buffer (pH 9): prepared by dissolving 2.1 g of sodium carbonate in 200 ml distilled water and 1.7 g of sodium bicarbonate in 200 ml distilled water and then mixing 10 ml of the carbonate solution with 115 ml of bicarbonate solution and completed to 500 ml [25]. Higher pH values (11–12) had been avoided to prevent hydroxylation of dansyl chloride [24].

p-chloranil was supplied from Sigma Aldrich Chemie GmbH (Steinheim, Germany) and freshly prepared 0.5% solution was prepared in DMF (solution b) [39]. Acetyl acetone: 8.4% *v*/*v* solution

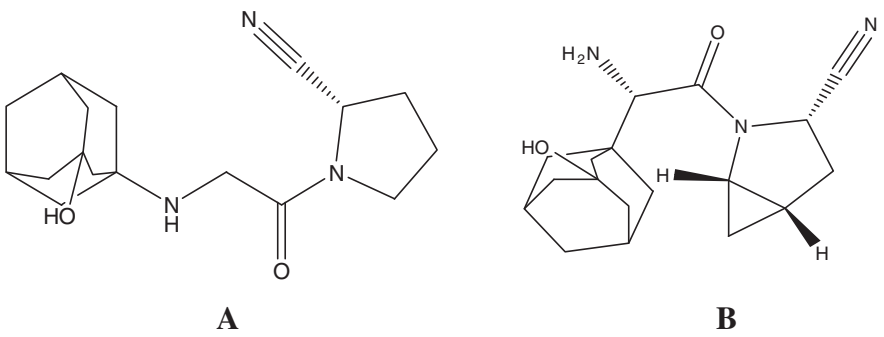

Fig. 1. Chemical structures of vildagliptin (A) and saxagliptin (B).