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Original Article

Utility of picric acid and 2,4 dinitrophenol as chromogenic reagents for visible spectrophotometric quantification of alogliptin

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ABSTRACT

Two simple and sensitive visible spectrophotometric methods (A and B) were developed for the determination of alogliptin in bulk and in its tablet dosage forms. The methods use the reaction of alogliptin with picric acid (method A) or 2,4 dinitrophenol (method B) in the chloroform medium. The complex of alogliptin with picric acid (method A) or 2,4 dinitrophenol (method B) showed λ_{max} at 415 nm and 430 nm respectively. The different conditions affecting the formation and stability of the complexes were optimized. The methods were validated statistically according to ICH. The calibration curve is linear over the concentration range of 10–60 μg ml^{-1} and 10–50 μg ml^{-1} for methods A and B, respectively. The proposed methods were successfully applied for the assay of alogliptin in tablets with good recoveries. Interference was not observed from common tablet excipients.

1. Introduction

Alogliptin (AGN), chemically described as 2-[[6-[(3R)-3-amino piperidin-1-yl]-3-methyl-2,4-dioxopyrimidin-1-yl] benzonitrile, is an oral antihyperglycemic agent of dipeptidyl peptidase-4 inhibitor class used in the treatment of type II diabetes milletus [1,2]. Usually dipeptidyl peptidase 4 degrades the glucagon like peptide 1 and incretins glucose-dependent insulinotropic polypeptide. The glucagon like peptide 1 and incretins glucose-dependent insulinotropic polypeptide stimulate glucose dependent secretion of insulin, repress glucose dependent glucagon secretion, reducing food intake and gastric emptying. The inhibition of dipeptidyl peptidase 4 by AGN increases the quantity of active plasma incretins and glucagon like peptide 1 that helps in glycemic control [3,4].

A few analytical methods have been proposed for the quantification of AGN. RP-HPLC [5–11] and HPTLC [12] methods have been developed for the quantification of AGN in bulk and tablet dosage forms. HPLC-MS/MS [13] and UPLC-MS/MS [14] methods have been reported to measure plasma AGN concentration in monkey and rat, respectively. Though the reported chromatographic methods are sensitive, they are expensive, time consuming, requires an expertise personnel and sophisticated HPLC techniques.

Spectrophotometry method of analysis is widely used in the estimation of drugs in pharmaceutical formulations owing to its simplicity, good sensitivity, cost effectiveness and easy availability. In the existing literature there are few reports regarding the use of spectrophotometry for the quantification of AGN in bulk and tablet dosage forms. Supriya et al., reported one UV spectrophotometric method for the estimation of AGN in raw material [11]. The method consisted of the measurement of absorbance of the methanolic AGN solution at 276 nm. Keyur et al., described UV spectrophotometric method for the quantification of AGN [15]. Water and methanol in the ratio of 50:50 (*v*/*v*) is used as solvent. Measurement of the absorbance of AGN in the above said solvent at 223 nm has served as the basis for the determination of AGN. Yadav et al., proposed a first-order spectrophotometry technique, in which 278 nm is chosen as λ_{max} for the determination of AGN [16]. The UV spectrophotometric methods are simple but they suffer from lack of selectivity as it involves measurements at shorter wavelength [11,15,16]. Raval & Srinivasa have proposed one visible spectrophotometric method for the quantification of AGN in formulations [17]. The reaction scheme involved is derivatization of AGN with 1,2-napthoquinone-4-sulfonic acid sodium in alkaline medium. The visible spectrophotometric method suffers from one or more disadvantages like less sensitive, lack of accuracy

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and precision, poor linear response [17]. Furthermore, the Raval & Srinivasa method was not fully validated according to ICH guidelines [17].

Picric acid and 2,4 dinitrophenol are used as analytical reagents for the quantification of substances of pharmaceutical significance bearing primary or secondary amine groups in its structure [18–21]. The reaction of AGN with picric acid or 2,4 dinitrophenol is not yet investigated. The objective of the present study was to develop cost effective, simple, sensitive and fully validated visible spectrophotometric methods (A and B) for the determination of AGN in pure and in its tablet dosage forms using picric acid and 2,4 dinitrophenol as analytical reagents. Methods A and B are based on the proton transfer from the picric acid (method A) or 2,4 dinitrophenol (method B) to AGN at room temperature and formation of yellow colored ion-pair complex in chloroform medium.

2. Experimental

2.1. Instrumentation

All spectrophotometric measurements were recorded with ELICO (Hyderabad, India) double beam model SL 159 digital spectrophotometer. One cm matched quartz cells were used for absorbance measurements.

2.2. Reagents

0.4% Picric acid (PCA) was prepared by dissolving 400 mg of PCA (Sdfine-Chem limited, Mumbai) in 100 ml chloroform (Merck, Mumbai). 0.1% 2,4 Dintrophenol (DNP) was prepared by dissolving 100 mg of DNP (Sd fine Chem Ltd., Mumbai, India) in 100 ml of chloroform (Merck, Hyderabad, India).

2.3. Standard solutions

Reference standard AGN was obtained as gifted sample from Rainbow Pharma Training Labs, Hyderabad, India. A stock standard solution containing 1 mg ml^{-1} of AGN was prepared in chloroform. Working standard solution equivalent to 100 μg ml^{-1} of AGN was obtained by appropriate dilution of stock solution with chloroform (methods A & B).

2.4. General assay procedure

2.4.1. Method A

To a set of 5 ml volumetric flasks, aliquot volumes (0.5–3 ml) containing the AGN (100 μg ml^{-1}) over the concentration range of 10–60 μg ml^{-1} were quantitatively transferred. The volume in all the flasks was made up to 3 ml with chloroform. A volume of 1 ml of 0.4% PCA solution was added to each flask. The solutions were mixed well and completed to the volume using chloroform. The flasks were left at room temperature for 10 min. The absorbance of yellow colored product was measured at 415 nm against a reagent blank prepared similarly omitting the drug.

2.4.2. Method B

Into a series of 5 ml volumetric flasks, volumes (0.5–2.5 ml) of AGN standard solution (100 μg ml^{-1}) equivalent to 10–50 μg ml^{-1} were transferred. The volume in all the flasks was made up to 2.5 ml with chloroform. To each flask, 1 ml of 0.1% DNP was added and brought up to the volume with chloroform. After 10 min, the absorbance of the yellowed colored product formed at room temperature was measured at 430 nm against the reagent blank prepared similarly omitting the drug.

For both the methods, calibration curves were constructed by plotting the absorbance against the final concentration of AGN. The corresponding regression equations were derived. The concentration of the unknown samples were read from the corresponding calibration graph or computed from the corresponding regression equation.

2.5. Procedure for the assay of AGN in tablets

Nesina tablets (Takeda Pharmaceuticals America, Inc., Deerfield) labeled to contain 6.25 mg/25 mg of AGN per tablet were purchased from the local pharmacy market. Twenty tablets were powdered and mixed thoroughly. A powdered amount equivalent to 100 mg of AGN was dissolved in 50 ml of chloroform by shaking continuously for 10 min. Whatmann no. 1 filter paper was used for filtering the solution. The filtrate was transferred into a 100 ml volumetric flask and made up to the volume with chloroform. This solution was appropriately diluted with chloroform. Convenient aliquots were subjected to analysis by the procedures described under methods A and B. The percentage recovery of the AGN was calculated from the corresponding calibration curve or regression equation.

3. Results and discussion

3.1. Basis of the reaction

The interaction of PCA or DNP with primary or secondary amine in organic solvent leads to the formation a yellow colored complex by proton transfer [22–25]. The proposed methods (A and B) are based on the formation of an ion-pair complex as a result of a proton transfer from hydroxyl group of Lewis acid, PCA (method A) or DNP (method B), to the amino group of Lewis base, AGN in chloroform medium. The resulted yellow colored ion-pair complexes showed maximum absorbance at 415 nm (method A) and 430 nm (method B) against the reagent blank. The absorption spectra of the yellow colored ion-pair complexes are shown in Figs.1 and 2. This reaction forms the basis for the visible spectrophotometric determination of AGN by the methods A and B. The probable reaction mechanisms are given in Schemes 1 and 2.

3.2. Optimization of the experimental conditions

The experimental conditions were established by studying the reaction as a function of the concentration of PCA (method A), DNP (method B) and the type of organic solvent as medium (methods A and B) for the maximum and stable color development.

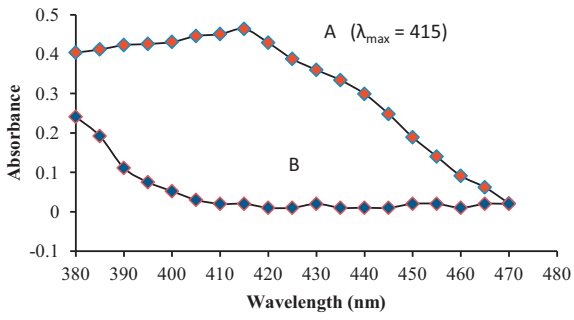

Fig. 1. Absorption spectra of (A) AGN-PCA ion-pair complex (B) Reagent blank (method A).

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