

Full Length Research Paper

Development of basic tests for sildenafil citrate and sildenafil citrate tablet

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The work involves the development of basic tests for sildenafil citrate (drug substance) and sildenafil citrate tablet (dosage form). The Basic Tests encompass physical observation, melting characteristics, colour and other test reactions for the drug substance and the dosage form. These tests are supplemented with thin layer chromatography. The test tube identification reactions were carried out to demonstrate the presence of the phenyl ether and sulphonamide functional groups as well as the N-substituted piperazine and pyrimidine rings on the molecule. The presence of sulphur and nitrogen in the drug moiety were also confirmed. The R_f values obtained from the chromatograms using three different solvent systems were found to be approximately same for sildenafil in the extracted form and the powdered tablet. A summary of test result for sildenafil citrate and sildenafil citrate tablet is presented that could be of use in quick verification of the identity of the drug.

Key words: Basic tests, sildenafil citrate, identity, verification.

INTRODUCTION

The basic concept of the pharmacopoeia is to provide monographs, which spell out standards and specifications that form a basis for the laboratory examination of drugs (WHO, 1979; Japanese Pharmacopoeia, 1998). The Basic Test Programme (BTP) is one of the recommendations of World Health Organization (WHO) Expert Committee on specification for pharmaceutical preparations which comprises simplified tests with the objective of providing readily available methods for verifying the identity of a drug substance or its dosage form using limited range of easily available reagents (USP, 1995; WHO, 1980). BTP is not intended to replace the requirements of the pharmacopoeia monograph. The pharmacopoeia gives an assurance of quality, while BTP

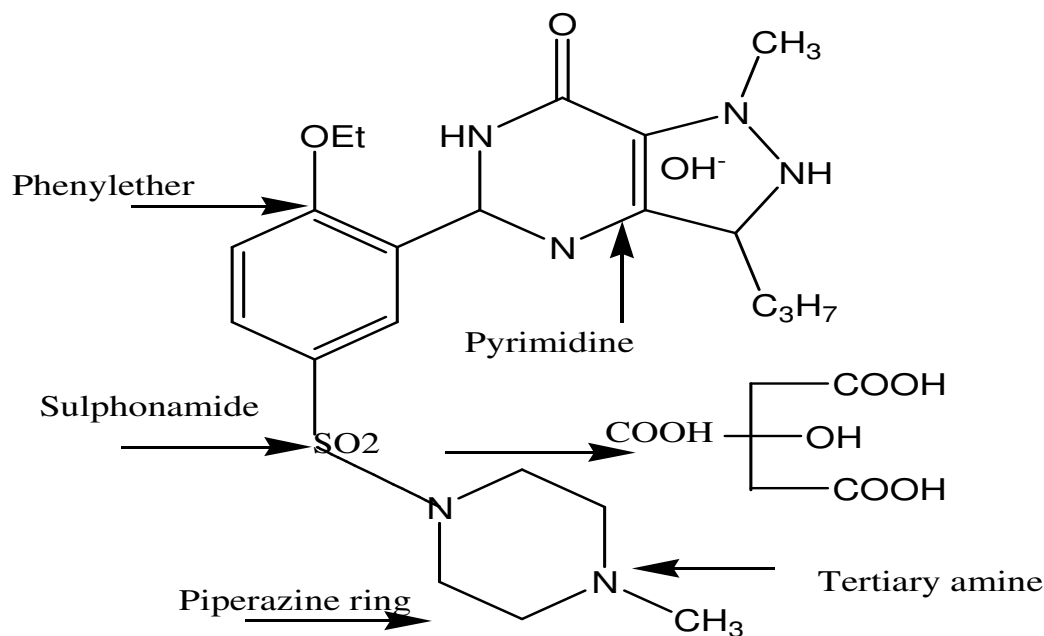
merely confirms identity (WHO, 1991). BTP was designed because of drug quality assurance problems occurring in developing countries which are worsened by lack of well-equipped laboratories, trained personnel and electricity outages (WHO, 1995, 1997). It also represents one of the many elements of quality assurance in the pharmaceutical supply system (Olaniyi, 2000a, b; Olaniyi and Adegbolagun, 1997). BTP essentially consists of three procedures namely physical observation, melting point characteristics and chemical tests supplemented by thin layer chromatography, TLC (WHO, 1997; Moffat, 1986; Pachaly, 1994).

BTP has been applied to drugs such as acetazolamide, a non bacteriostatic sulphonamide indicated for glaucoma and non glaucomatous conditions (WHO, 1980, 1991). The current challenges posed by fake, counterfeit and adulterated drugs make the need for the extensive use of BTP imperative (Olaniyi and Idowu, 1993).

Sildenafil citrate was produced in response to the social problem affecting an estimated figure of 140 million men worldwide presenting with erectile dysfunction that may lead to impotence (Adeneye, 2000). It inhibits the enzyme

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Abbreviation: BTP, Basic Test Programme; TLC, Thin layer chromatography.



1-[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl)-1H-pyrazolo-[4,3-d]pyrimidin-5-yl]-4-ethoxyphenyl]-sulphonyl-4-methylpiperazine

Figure 1. Structure of sildenafil highlighting the functional groups in the molecule.

phosphodiesterase PDE5 which is responsible for the degradation of cGMP in the corpus cavernosum. Smooth muscle relaxation and increased flow of blood to the penis is associated with increased levels of cGMP. Research has shown that derivatives of pyrazole {4,3-d} pyrimidine-7-one of which sildenafil is a prototype gives potent cGMP PDE5 inhibition (Figure 1). No report on the availability of fake sildenafil tablets but the high cost coupled with the social and biological usefulness of the drug could make it a target drug for counterfeiters.

MATERIALS AND METHODS

Materials

Sildenafil citrate (Viagra®) 100 mg tablets by Pfizer USA; batch number 99R49A; manufacturing and expiring dates 05/2000 and 05/2004 respectively were purchased from a registered pharmaceutical shop in Uyo, Nigeria.

Sulphuric acid, hydrochloric acid, nitric acid sodium hydroxide, formaldehyde, ethyl acetate, copper sulphate, ammonia, potassium permanganate and sodium bicarbonate were all of analytical grade, products of Nanjing chemical reagent company China. TLC silica GF₂₅₄ plates (5 x 10 cm) were obtained from Biolife Chemical Limited, Nigeria.

Methods

Test solution preparation

5 x 100 mg tablets of sildenafil citrate were crushed to release the

powdered substance from the blue coating. The crushed granules were further grinded to fine powder. 250 mg of powder was dissolved in 100 ml of methanol and filtered. The filtrate was evaporated on water bath to give crystals of sildenafil citrate. This was dried in an oven at 50 °C for 15 min (Olaniyi and Ogungbamila, 1998). This was referred to as test substance 1 while the powdered tablet as test substance 2. Weighed quantities of test substance 1 and 2 in appropriate solvents formed the test solutions.

Prepared reagents

Reagent A was prepared by adding 10 ml of saturated solution of mercurous nitrate to 20 mg of sodium bicarbonate (Moffat, 1986). The effervescence ceased and the precipitate formed turned yellow and then to biscuit colour. Reagent B was prepared by adding 1 ml of formaldehyde to 9 ml of sulphuric acid. Reagent C was prepared by dissolving 0.1 g of mercury chloride in 16 ml of water. Reagent D was prepared by weighing out 5.0 g of sodium nitrate in a conical flask and 50 ml of concentrated sulphuric acid was added.

Colour reactions

Test for sulphonamide group: 100 mg of test substance 1 and 2 were dissolved separately in 5 ml of ethanol. A drop of reagent A was added at intervals of 2 min and shaken up for 10 min. A blank of ethanol was similarly treated and the obtained colour was noted. Secondly, 100 mg of test substance 1 and 2 were dissolved separately in 10 ml of 0.1 M sodium hydroxide. Copper sulphate (10% w/v) was added drop wise to the solutions.

Tests for N substituted piperazine: 20 mg of test substances 1 and 2 were dissolved separately in 2 ml of water. 1 ml of reagent C was added followed by 8 drops of 2 M sulphuric acid. This was heated to boil and potassium permanganate (10 g/L) was added drop wise.

Table 1. The phenomena of the colour reactions of sildenafil citrate and sildenafil citrate tablet.

Reaction	Test substance	Observation	Inference
Reagent C	1	Yellow colour	Phenylethyl group suspected
Reagent C	2	No colour	Interference due to excipient
Reagent C with heat	1	Brown colour	Phenyl ethyl group confirmed
	2	No reaction	Interference due to excipients
Reagent A with ethanol	1	Grey colour	Sulphonamide group suspected
	2	Brown colour	Sulphonamide group suspected
Blank ethanol	1	No reaction	
	2	No reaction	
0.1M Sodium sulphate and 10% copper sulphate	1	Light green colour	Presence of sulphonamide
	2	Light green colour	Presence of sulphonamide
Reagent B with 2 ml sulphuric acid and potassium permanganate(10 g/l)	1	No reaction	Presence of tertiary amine
	2	No reaction	Presence of tertiary amine
Sodium nitrite and 2ml hydrochloric acid	1		Tertiary amine is confirmed.
	2		Tertiary amine is confirmed

Secondly, 20 mg of test substances 1 and 2 were dissolved separately in 0.5 ml of 2 M hydrochloric acid. 50 mg of sodium nitrite was added to the solutions. The solutions were cooled in ice with stirring.

Test for pyrimidinyl ring: 100 mg of test substances 1 and 2 were put separately on a white tile. A drop of reagent B was added to the test substances.

Test for Phenyl ether group: 3 drops of reagent D was added to 100 mg of test substances 1 and 2 on a white tile 20 mg of test substances 1 and 2 were put into separate test tubes and 1.5 ml of reagent D was added to each test tube and heated at 100°C in a water bath.

Sodium fusion test

50 mg of test substance 1 was put in a dry test tube. A piece of sodium metal was added and heated to dull red heat for 1 min. On cooling, 3 ml of water was added, boiled and filtered. The filtrate was divided into 3 fractions a, b and c. To 1 ml of filtrate (fraction a) was added few crystals of ferrous sulphate and boiled for 30 sec. The solution was acidified with 3 drops of sulphuric acid. 1 ml of filtrate (fraction b) was acidified with 3 drops of acetic acid and 6 drops lead acetate solution was added. The mixture was boiled for 15 min. 2 ml of the filtrate (fraction c) was put in test tube and few crystals of ferrous cyanide were added. The solution was boiled and cooled and acidified with 3 drops of sulphuric acid.

Melting point determination

The melting point was determined using the extracted powder.

TLC Identification

TLC identification was carried out using the following chromatographic conditions; Sample solutions: i) 20 mg of test

substance 1 dissolved in 5 ml of methanol; ii) 40 mg of test substance 2 dissolved in 5 ml of methanol. Stationary phase: GF₂₅₄ Mobile phase: i) Methanol: chloroform (4:1, v/v); ii) Methanol: ethyl acetate (4: 1, v/v) iii. Methanol: ethyl acetate: ammonium hydroxide (2:8:1). Visualization: i) UV light; ii) Iodine tank.

RESULTS

Physical observation

Sildenafil is a white to off white crystalline powder that is odourless and tasteless.

Heating and melting behaviour

The pure drug melted between 182 to 186°C with decomposition turning brown. The pure drug melted and sublimed with soot leaving a black residue without any characteristic odour (Adeneye, 2000).

Colour reaction

The observed reaction of sildenafil citrate (drug substance) and sildenafil citrate tablet (Dosage form) are outlined in Table 1

Sodium fusion test

The outcome of the sodium fusion test is expressed in Table 2.

Table 2. Result of sodium fusion test using pure drug.

Test	Result	Inferences
Sodium metal in methanol and filtered	No reaction	
Filtrate with nitroprusside	Purple colour	Presence of sulphur
Filtrate with acetic acid and lead acetate	Black precipitate	Presence of sulphur is confirmed
Filtrate with ferrous sulphate crystals and sulphuric acid	Prussian blue precipitate	Presence of nitrogen

TLC profile.

Table 3. R_f values for the pure drug and the powdered sildenafil citrate tablet in the various solvent systems.

Solvent system	R_f value for pure drug	R_f value for powdered tablet
Methanol: Chloroform (4:1,v/v)	0.68	0.68
Methanol : Ethylacetate (4:1,v/v)	0.74	0.75
Methanol: Ethylacetate: Ammonium hydroxide (2:8:1,v/v)	0.80	0.81

Thin layer chromatography

The result of the chromatographic determination of the drug substance and the powdered dosage form in the different solvent systems employed are expressed in Table 3

DISCUSSION

One spot was located on the chromatograms for each of the spotted test substances. This indicated that sildenafil is present in a pure form and no decomposition has occurred. The observed R_f values of the spots for the test substances in each solvent system were approximately the same which further confirms the identity and purity of the drug substance.

The melting point of the extracted drug was 182 to 186°C as against the value obtained from literature being 191 to 202°C (Adeneye, 2000). The low melting point is attributable to incompleteness of the extraction process. The test tube reactions were performed and observed based on the four functional groups present on sildenafil moiety. Two tests were performed for the piperazine ring giving negative results. Positive results are expected for molecules with piperazine ring having free amino nitrogen. N- alkyl substitution confers inactivity on the piperazine ring.

Sulphonamides are hydrolysable in sodium hydroxide hence it was used to dissolve the test substance before 10% solution of copper sulphate was added drop wise in the copper sulphate test for sulphonamide. Nucleophilic attack on the sulphonyl sulphur gives green colouration from the initial blue. The second test for sulphonamide involving the use of mercurous nitrate and sodium bicarbonate required the use of ethanol as the solvent so as to increase the speed of reaction. Sulphonamides with

additional rings react faster in reaction medium containing ethanol.

The test for phenyl ether required vigorous reaction condition involving heat at 100°C to get a positive result as ethers are known to be generally uncreative compounds. Ethers are stable towards bases, oxidizing and reducing agents but undergo acid cleavage under high temperature. Sulphuric acid in its concentrated form will dissolve unsaturated hydrocarbons, bases and most oxygen containing compounds. Sulphuric acid in the presence of sodium nitrite reacts with the phenyl ether group producing a yellow colour. The colour was however masked by the excipients present in test substance 2.

Conclusion

In the work presented, the basic tests for sildenafil citrate tablet and the drug substance has been developed by visual inspection, melting characteristics and TLC methods. It has been demonstrated that the functional groups present in the molecular structure of sildenafil citrate can be exploited to identify the drug. The developed basic tests are sufficient to verify the identity of the drug in circumstances where fully equipped laboratories are not available employing limited volume or quantity of reagents. The developed test should be investigated further under various environmental conditions.

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