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Physicochemical Compatibility and Stability of Reconstituted Fluconazole in Mini-Containers

Omotoso, Abayomi Ebenezer*, Ezealisiji Kenneth Maduabuchi, Ibekwe Victoria Kelechi
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria.

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***Corresponding author**
Omotoso, Abayomi Ebenezer

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Abstract: Multiple Intravenous drug administered in one route cannot be avoided in critical care as the need for various drug is higher than the amount of venous access, therefore this study is aimed at assessing the physicochemical compatibility and stability of fluconazole in infusion fluids over time. Two different brands of fluconazole infusion were reconstituted with Ringer's lactate solution (400 µg/mL), 5% Dextrose water (400 µg/mL) or Sodium bicarbonate injection (2mg/mL) in a separate polyvinyl chloride containers. The reconstituted samples were visually inspected for colour, odour changes, presence of particulate matter, and then assayed using a UV-Visible Spectrophotometric method in triplicate at time 0, 0.5, 1, 2, 8, and 24 hrs. The pH was further monitored at predetermined time interval. The reconstituted samples showed no colour or odour change and no particulate matter. The concentration of un-degraded fluconazole in the reconstituted samples remain > 90% of the initial amount (t = 0) Thus, indicating no physical interaction between fluconazole, infusion fluids and the packaging material. The results obtained in this study indicate that infusion of reconstituted fluconazole with any of the infusion fluid used in this study is safe, stable and compatible within the 24 hr experimental period.

Keywords: Compatibility, Stability, Fluconazole, pH, infusion fluids.

INTRODUCTION

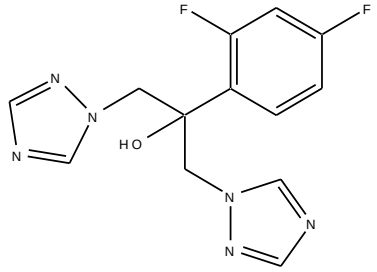
Intravenous (IV) therapy is complex, potentially dangerous and error prone, thus the need for strategies to reduce the risk and complications. Infusion therapy through intravenous access is a therapeutic option used in the treatment of many hospitalized patients. Infusion medications are associated with high risk of harm.

Once injected, reversal is almost impossible unless an antidote exists. The intravenous route of medication administration has many advantages and benefits. The most important is the immediate therapeutic effect of medications [1]. Thus, IV route is the preferred route for critically ill patients. However, there are a lot of possible direct and negative side effects. There have also been reports of death and harm following medication errors. Thus, the primary focus should be to identify IV therapy associated drug-related problems (DRPs) [2].

Drug-therapy related problem can be defined as an event or circumstance involving drug treatment that actually or potentially interferes with the patient experiencing an optimum outcome of medical care. One of the causes of DRPs during intravenous administration is incompatibilities of the drugs. The US National Coordinating Committee on Large Volume Parenteral defines incompatibility as a phenomenon which occurs when one drug is mixed with another drug to produce, by physicochemical means, a new product unsuitable for administration to the patient due to some modifications [2]. However, the common concept of incompatibility occurs in an *in vitro* setting in regard to the mixing of multiple medications through a single infusion line, single container or single syringe, while venous access is limited [3]. Furthermore, the definition of incompatibility is often confused with that of instability. However, there is agreement that instability is more about an unstoppable degradation process attributable to storage conditions [4]. Driscoll [5] extended the definition of instability thus "a process of deterioration or degradation that changes pharmaceutical and pharmacological reactions, while incompatibility is more about interactions arising out of co-administration". In fact, incompatibility ultimately results in effects that are similar to those of instability.

Administering incompatible drugs through the same intravenous line often result in negative consequences and even death. Physical, chemical and therapeutic incompatibility are incompatibilities often associated with IV administration. Stability is defined

as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the re-test or expiration dating periods. Due to their possible composition, pharmaceuticals are especially sensitive to environmental factors. Strict storage conditions are necessary for the maintenance of integrity and product activity. Stability testing of an active substance or finished product provides evidence on how the quality of a drug substance or drug product varies with time influenced by a variety of environmental factors such as temperature, humidity and light. The results from stability testing are applied in developing a suitable manufacturing process, selecting proper packaging, storage conditions, product's shelf life and expiration dates [6]. Stability of pharmaceutical formulations are usually influenced by pH, surfactants, solubility, temperature, oxygen, light and packaging materials [7].



2-(2,4-difluorophenyl)-1,3-bis (1H-1,2,4-triazole-1-yl)propan-2-ol

Fluconazole whose systematic name is 2-(2, 4-diflurophenyl)-1, 3-bis (1*H*-1, 2, 4-triazol-1-yl)-2-propanol is a synthetic triazole derivative antifungal agent found to be effective against a wide range of systemic and superficial fungal infections. Fluconazole solution has a pH of 4.0 - 8.0 while the solid sample melting point range is 138 – 142°C. It's slightly soluble in water but soluble in organic solvents such as chloroform, propylene glycol, alcohol, and polyethoxylated castor oil [8]. Fluconazole is well absorbed after oral administration, often a plasma level of over 90% (systemic bioavailability) can be achieved after intravenous administration. Mammalian demethylase activity is much less sensitive to fluconazole than fungal demethylase. This inhibition prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols [9]. Fluconazole is administered at the dose of 200 mg – 400 mg daily for adults and 6 mg/kg – 12 mg/kg for children (the IV and oral dosages are the same). Intravenous infusion should be administrated at a rate not exceeding 10ml/minute. In children, the rate should not exceed 5 mL/min. for premature infants infusion time should not be less than 15 minutes. Fluconazole solution for infusion is formulated in Sodium chloride 9 mg/mL (0.9%), each 200 mg (100 mL bottle) containing 15 mmol each of Na^+ and Cl^-. Studies carried on the degradation of Fluconazole shows that it undergoes oxidative degradation to 2-(2, 4-difluorophenyl)-1-(1H-1, 2, 4-triazol-1-yl) propan-2-ol and 1-methyl-1, 2, 4-triazole in the presence of hydrogen peroxide [10].

Unfortunately, the common published data sources are often not suitable for hospital conditions. The established literature often contains insufficient details. Fluconazole are often reconstituted with Ringers lactate, 5% Dextrose solution or Sodium bicarbonate injections [12]. If *"in –use"* stability information is not available, stability based on practical consideration should be developed. This poses the question investigated by this study: can the stability of this medication be assured over certain storage period before administration, especially when branded formulations are diluted? This step determines whether the routine reconstitution of Fluconazole infusion is safe in terms of compatibility. The stability of the reconstituted Fluconazole infusion within 24 hr was determined in order to properly advice the health care providers in clinical settings on the status of pre-mixed Fluconazole [11].

Materials

Fluconazole pure sample (kind gift from 'Drugfeild Nigeria), Juconazole® 2 mg/ml (Juhel, 'Nigeria), 'Flucanir® 2 mg/ml (Nirlife, 'Nigeria), Ringer's Lactate Solution, (Juhel Nigeria), Dextrose Water 5%w/v (DANA, Nigeria.), Sodium Bicarbonate injection BP 8.4%w/v (Martindale, UK), Methanol Analar Grade (Guanghua Sci-Tech Co., China), UV-VIS Spectrophotometer (Techmel & Techmel, USA), Volumetric Flask (10 mL, 25 mL, 50 mL) (Pyrex, England), Analytical Weighing balance (Ohaus, Japan), Filter Paper (Whatmann #1), pH Universal meter (PEC Medical, USA), Pipette and Beakers.

METHOD

Research Settings

The laboratory experiments were conducted *in-vitro*, thus the issue of ethical approval does not arise.

Study Design

This research was designed to evaluate the physical and chemical compatibility of pre-mixed Fluconazole. This study was carried out under ambient temperature, humidity and light exposure. The two brands of fluconazole under study were separately diluted with Ringer′s lactate solution, 5% Dextrose solution and Sodium bicarbonate injection in 500 mL minibags and assayed in triplicate.

Preparation of Standard Stock Solution

Accurately weighed 10 mg of Fluconazole was transferred into 100 mL volumetric flask containing 30 mL of methanol. This was sonicated for 10 minutes, and made up to the mark with methanol. (100 µg/mL)

Scanning and Determination of Wavelength of Maximum Absorption

Scanning for wavelength of maximum absorption was carried out using stock solution of pure Fluconazole using UV-VIS Spectrometer within the range of 200-800 nm.

Preparation of Serial dilutions

Serial dilutions of the stock solution (100 µg/mL) with methanol was carried out to prepare the working standards within the range of 2-16 µg/mL. The absorbance values of above solutions were measured at the determined λ_{max} (260 nm) against methanol as blank and the calibration curve was prepared.

Sample Preparation for Reconstituted Samples

The solution of commercially available Juconazole® (100 mL) was mixed with 400 mL of commercially available Dextrose solution (5%), in polyvinyl chloride minibags to obtain the reconstituted solution (400 µg/mL). The above procedure was repeated for Ringer's lactate. A 0.25 mL volume each of the above reconstituted solutions was made up to mark with methanol in a 25 mL volumetric flask. Ten millilitre (10 mL) of a commercially available Sodium bicarbonate injection was diluted to 100 mL with Juconazole® from which 0.1 mL was further diluted to 100 mL with methanol to obtain the reconstituted stock solution (840 µg/mL). A 1.0 mL of the stock solution (840 µg/mL) containing Juconazole® and Sodium bicarbonate injection were made to mark in a 100 mL volumetric flask with methanol in triplicate.

The reconstituted Fluconazole samples were stored in the 500 mL minibags in an open room under ambient light, temperature and humidity. The samples were assayed using UV/Vis Spectrophotometer at 0, 0.5, 1, 2, 8 and 24 hr in triplicate and the concentrations determined from the calibration curve. The above procedures were repeated for IV Flucanir®.

Determination of pH

The pH of the reconstituted samples were taken with pH meter (which was calibrated prior to use) at time 0, 0.5, 1, 2, 8 and 24 hr. The diluted solutions were monitored for changes in pH. If the pH change is more than half amount or the pH shift beyond the usual range specified by the manufacturer, this will be taken as an indication that there might be a potential problem.

Determination of Odour, Colour, and Particulate (visible) Matter

Physical incompatibility was visually evaluated to assess clarity, colour changes and odour. The observations were made independently by two people using a black background and a white background under fluorescent lamp. Colour changes were more easily noticed against a white background, while clarity was more easily observed against a black background to demonstrate haziness or precipitation. The solution was considered incompatible physically if any presence of discolouration or haziness was visible.

RESULTS

Pharmaceutical product is of vital importance for patient's safety. The presence of impurities may influence the efficacy and safety of pharmaceuticals especially parenteral. Impurities and potential degradation products can cause changing of chemical, pharmacological and toxicological properties of drugs thus, having significant impact on product quality and safety. Fluconazole infusion being a pharmaceutical product needs to maintain its purity, identity, safety and efficacy in order to maintain patient's safety and achieve optimum treatment outcome.

The tables and figures below are the results obtained after assaying Fluconazole both in its pure form and the reconstituted form:

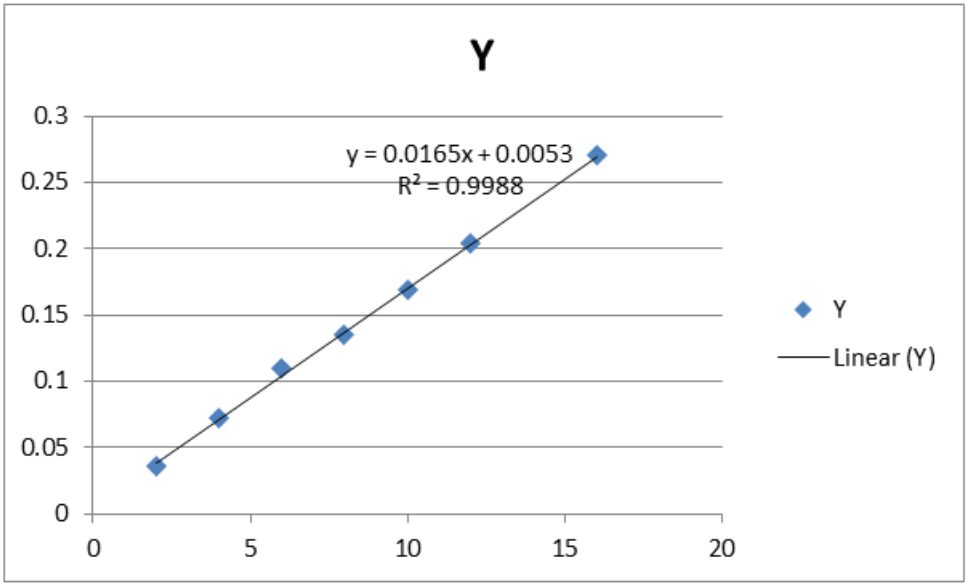


Fig-1: Calibration curve for the determination of Fluconazole at λ_{max} = 260 nm

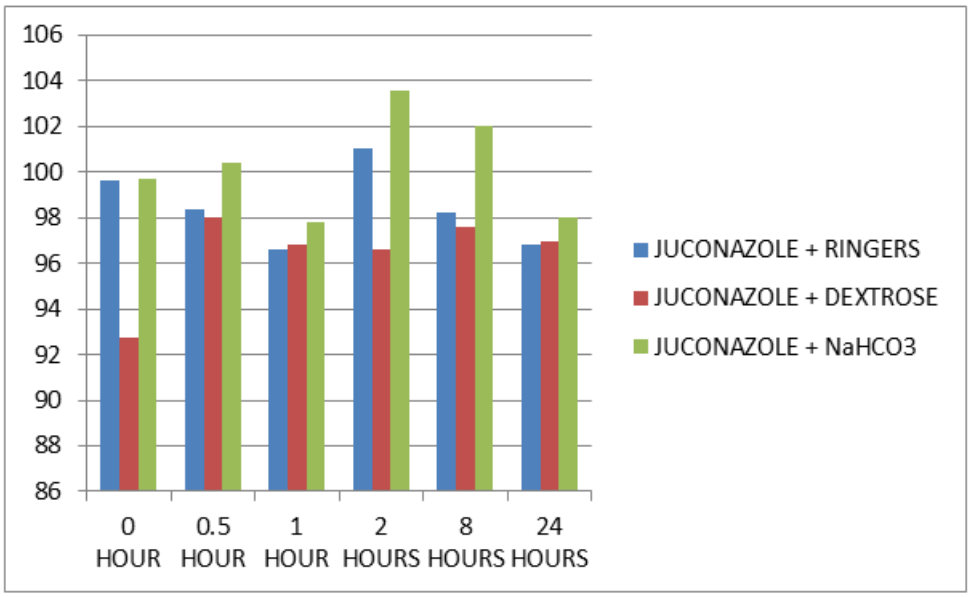


Fig-2: Percentage Fluconazole in the Reconstituted Juconazole® within 24hr at ambient temperature

Table-1: Estimated Percentage of initial amount of Fluconazole in the Reconstituted Juconazole® within 24 hr.

	Juconazole® + Ringers Lactate				Juconazole®+ 5% Dextrose				Juconazole®+ $NaHCO_3$			
Time (hr)	**Mean Abs.**	**SD**	**Conc. µg/mL**	**% A**	**Mean Abs.**	**SD**	**Conc. µg/mL**	**% A**	**Mean Abs.**	**SD**	**Conc. µg/mL**	**% A**
0.0	0.170	0.004	9.982	99.75	0.158	0.001	9.275	92.75	0.160	0.003	9.38	99.7
0.5	0.168	0.001	9.840	98.40	0.167	0.001	9.800	98.00	0.161	0.002	9.44	100.4
1.0	0.165	0.001	9.659	96.59	0.165	0	9.679	96.79	0.157	0.001	9.20	97.8
2.0	0.176	0.002	10.103	101.03	0.165	0.001	9.659	96.59	0.166	0.01	9.74	103.6
8.0	0.167	0.001	9.820	98.20	0.166	0.002	9.760	97.60	0.164	0.001	9.60	102.0
24.0	0.165	0.001	9.679	96.79	0.165	0.002	9.699	96.99	0.158	0.001	9.23	98.0

Where: % A = Percentage of initial amount of drug in solution; Abs. = Absorbance.

Table-2: Estimated Percentage of initial amount of Fluconazole in the Reconstituted Flucanir® within 24 hr.

	Flucanir®+ Ringers Lactate				Flucanir®+ 5% Dextrose				Flucanir® + $NaHCO_3$			
Time (hr)	**Mean Abs.**	**SD**	**Conc. µg/mL**	**% A**	**Mean Abs.**	**SD**	**Conc. µg/mL**	**% A**	**Mean Abs.**	**SD**	**Conc. µg/mL**	**% A**
0.0	0.162	0.001	9.477	94.77	0.167	0.001	9.820	98.2	0.162	0.007	9.51	101.2
0.5	0.169	0.002	9.962	99.62	0.166	0.001	9.739	97.39	0.165	0.001	9.66	102.8
1.0	0.164	0.001	9.600	96.00	0.169	0.009	9.941	99.41	0.163	0	9.55	101.0
2,0	0.167	0.009	9.800	98.00	0.167	0.006	9.800	98.00	0.161	0.002	9.44	100.4
8.0	0.166	0.002	9.719	97.19	0.165	0.001	9.659	96.59	0.158	0.001	9.23	98.0
24.0	0.169	0.009	9.941	99.41	0.167	0.008	9.820	98.20	0.164	0.001	9.60	102.0

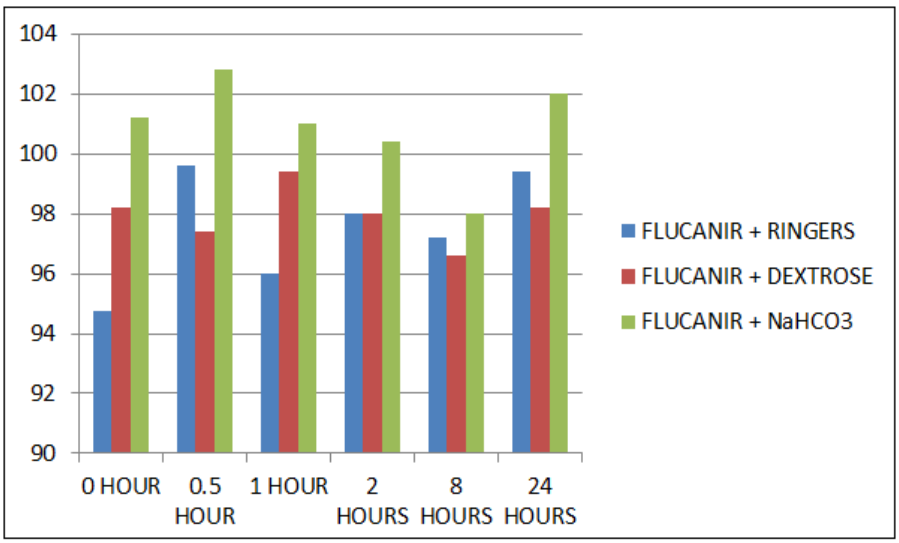


Fig-3: Percentage of Fluconazole concentration in the Reconstituted Flucanir® within 24 hr at ambient temperature

Physicochemical Analysis

Experiments were carried out to determine the physicochemical characteristics of IV Fluconazole and the infusion fluids. The physicochemical characters analyzed include pH, colour of vial content and colour of reconstituted solution. The results obtained from these tests are expressed below:

Table-3: Physicochemical characteristics pre-reconstitution of samples

Sample	Colour	Odour	pH
Juconazole®	Colourless	Odourless	5.77 ± 0.004
Flucanir®	Colourless	Odourless	5.78 ± 0.001
Ringers lactate	Colourless	Odourless	6.70 ± 0.007
Dextrose 5%	Colourless	Odourless	6.25 ± 0.003
Sodium bicarbonate	Colourless	Odourless	7.75 ± 0.001

Table-4: Physicochemical characteristics post-reconstitution of samples

Reconstituted sample	Colour	Odour	pH
Juconazole® +Ringer's	Colourless	Odourless	5.89 ± 0.002
Juconazole® + Dextrose	Colourless	Odourless	4.38 ± 0.002
Juconazole® + $NaHCO_3$	Colourless	Odourless	8.12 ± 0.008
Flucanir® + Ringer's	Colourless	Odourless	6.31 ± 0.001
Flucanir® + Dextrose	Colourless	Odourless	4.06 ± 0.005
Flucanir® + $NaHCO_3$	Colourless	Odourless	8.18 ± 0.001

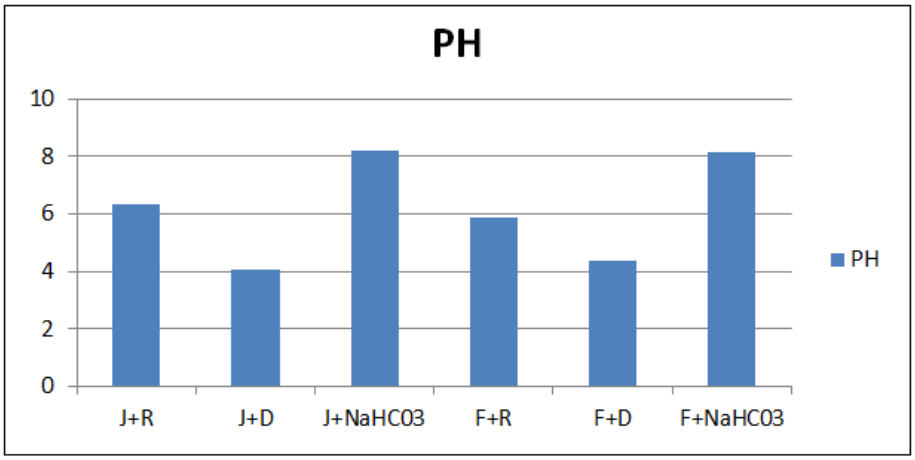


Fig-4: pH variation of the reconstituted Fluconazole in Ringers lactate (R), Dextrose solution (D) and Sodium bicarbonate in minibags during 24 hour under ambient room temperature.

Particulate matter testing

Table-5: Number of Visible Particles observed before Sample Reconstitution

Samples	White particles	Black particles
Juconazole®	Nil	Nil
Flucanir®	Nil	Nil
Ringer's Lactate	Nil	Nil
Dextrose solution	Nil	Nil
Sodium bicarbonate	Nil	Nil

Table -6: Number of visible particles after Sample Reconstitution

Reconstituted samples	White particles	Black particles
Juconazole® +Ringer's	Nil	Nil
Juconazole® + Dextrose	Nil	Nil
Juconazole® + $NaHCO_3$	Nil	Nil
Flucanir® + Ringer's	Nil	Nil
Flucanir® + Dextrose	Nil	Nil
Flucanir® + $NaHCO_3$	Nil	Nil

DISCUSSION

To achieve a precise dose, some intravenous (IV) medication need dose manipulation by dilution, reconstitution and titration through micro-infusion. When the IV drug has been diluted, the manipulation has the potential to change the compatibility and stability of the original formulation [1]. Reconstituted IV medication in minibags should also be compatible physically and chemically, in addition, they should be stable during storage and administration [3]. Although, based on previous findings, sedatives, analgesics and inotropes were found to be the most common medications reconstituted into minibags to manage dosage [1], but in developing nations, due to poor hygiene and thus the prevalent of infectious diseases, medications for communicable diseases also belongs to the above categories. These medications were often administered through slow continuous infusion with titration of the dose. Often these reconstitutions were completed prior to administration during any spare time in nursing work station. Sometimes the minibags were reconstituted by nurses in charge of the previous shift and stored at room temperature under the diffuse light of the ward environment. This may lead to potential incompatibility and instability of the medications in the clinical settings.

Some factors are related to compatibility and/or stability of Fluconazole. According to International Conference on Harmonization (ICH) guidelines which classified the current research as a stability study with special conditions [4]. The ICH defines stability as meeting the acceptance criteria for appearance, physical characteristics and functionalities (ICH Guideline); having no degradation peak and change within 10 % of initial concentration.

All samples (both reconstituted and unreconstituted) were visually clear, presenting neither colour change nor precipitate formation and were also free of particulate matter within the 24 hr period of the study (Table-3.3 and Table-3.4). Chemical incompatibility is an irreversible change that can be apparent as a pH or concentration as well as change in the drug from active to inactive or toxic form [4].

As shown in Tables 3.3 and 3.4, the pH levels of the tested samples were acidic before and after reconstitution except for that of sodium bicarbonate whose alkalinity increases in the reconstituted samples. This was expected, because reaction of sodium bicarbonates with fluconazole is expected to produce a resultant alkaline solution, though the pH is within the specified pH range for fluconazole infusion. Fluconazole in stable in acidic solution, this account for its formulation in an acidic solution. Thus it is imperative that pH is an essential determinant of Fluconazole stability. In the laboratory, the pH of the undiluted Fluconazole ranged from 5.77-5.78, but when reconstituted with Dextrose water, the pH decreases to 4.38-4.06, although the change in pH is significant ($p < 0.05$), but still within the acceptable limit; when reconstituted with Ringers lactate solution the change in pH was insignificant. A wider range of pH values appeared for Flucanir® and Dextrose after 24 hours, also within the acceptable limit. A more significant change in pH was observed on addition of Sodium bicarbonate to the Fluconazole preparation (pH = 8.18 - 812). Although this was expected, sodium bicarbonate being a weak base will combine with the hydroxyl group of the Fluconazole to produce more basic solution (the basicity of fluconazole maybe due to one of the six nitrogen atoms in the molecule). Fluconazole degradation has been shown to be pH-dependent [9]. Change in pH indicated a change in the degree of ionization. Based on Henderson-Hasselbalch equation, pH change of 1 unit can convert 10 fold concentration of ionized entity to unionized form.

In addition, chemical compatibility was also evaluated using the percentage of each drug remaining relative to zero (0) time during the 24 hr period. As demonstrated in the results depicted by the graph Figure 3.2 and 3.3, the amount of drug remaining were consistently greater than 90 % during the 24 hours period of the study. This indicated that fluconazole do not degrade in the presence of these electrolytes.

The initial pH values of the samples were consistent with the acceptable pH ranges specified in the USP (2005), that is, pH of 6.0 – 7.5 for Ringer's lactate, pH 3.5 – 6.5 for 5% Dextrose, pH 7.0 – 8.5 for Sodium bicarbonate injection and pH of 4.0 – 8.0 for Fluconazole. There was no considerable change in pH observed throughout the study in the reconstituted samples of Fluconazole infusion (Table-3.4). This indicates that Fluconazole is stable in the different infusion fluid studied. Based on these findings, the routine hospital procedure of reconstituting Fluconazole with Ringers lactate solution, Dextrose 5 %w/v solution, and Sodium bicarbonate were chemically safe. This study indicated that the commercially available Fluconazole samples studied retained more than 90 % of the initial amount within the 24 hr of this study and thus the storage time of Fluconazole when reconstituted can be extended even beyond 24 hr when administered within the hospital settings for centralized preparation in the hospital pharmacy.

CONCLUSION

This study concludes that the reconstituted Fluconazole is both stable -in and compatible -with the infusion solutions under study but the reconstituted Fluconazole with Sodium bicarbonate is the least stable among the infusion solutions. Thus, Fluconazole reconstituted with Sodium bicarbonate should be administered within 24 hr after reconstitution.

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