ADMIXTURE STABILITY STUDIES OF ZIDOVUDINE AND SELECTED PHARMACEUTICALS

by

PATRICK MUSAMI

(Under the Direction of DR ETHAN WILL TAYLOR)

ABSTRACT

The stability of 10mg/mL zidovudine and either 12.5mg/mL dobutamine or 25 mg/L ranitidine hydrochloride stored in 0.9% sodium chloride and 5 % dextrose injections in 50 cc polyvinyl chloride (PVC) bags stored at 4 degrees Celsius and ambient temperature up to 24 hr was studied. Samples were analysed at 0, 4, 8, and 24 hr after preparing the controls and known mixtures, using a reversed phase stability indicating HPLC assay. The drug mixtures were stable up to 24 hrs as shown by the % remaining drug concentrations above 90 % at the respective sampling times.The pH of the drug solutions remained constant over time. These data support unequivocally the stability of zidovudine/dobutamine or zidovudine/ranitidine admixtures under the storage conditions studied in this investigation.

INDEX WORDS: PVC , HPLC, zidovudine, dobutamine, ranitidine, ambient temperature, and 4°C.

STABILITY INDICATING STUDIES OF ZIDOVUDINE AND SELECTED PHARMACEUTICALS

by

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DEDICATION

This is dedicated to all those who supported my quest for further education.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Hospital pharmacists are frequently asked to prepare special formulations that are not commercially available. These medications may be administered concurrently as admixtures, and could be for immediate use or for storage. This usually entails addition of drugs to parenteral and dialysis solutions, and special topical or oral preparations such as ointments, eye drops, syrups, or suspensions. Before this can be done, the pharmacist needs to ensure that the drug product is stable under storage conditions. It is therefore necessary to do stability studies of such a mixture to assign a shelf life (1-3). On the other hand, drug manufacturers view stability testing of pharmaceutical products as yet another "red tape" that must be overcome before marketing the drug (4). Recently, the International Conference on Harmonization (ICH) has issued guidelines on stressed storage conditions for pharmaceutical products, but prior to this there were inconsistencies in the stability testing criteria. The tests should cover the features susceptible to change during storage and likely to influence quality, safety, and or efficacy. Analytical procedures should be fully validated and the assays should be stability indicating. The range of testing should cover not only chemical stability, but also loss of preservative, physical properties, organoleptic properties and, where required, microbiological attributes. Preservative efficacy and testing and assays on stored samples

should be carried out to determine the content and efficacy of any antimicrobiological preservatives (5- 7).The stability of a drug is often expressed in quantitative terms such as shelf life. Provided that degradation products are not toxic, a preparation is generally considered acceptable if it mantains at least 90 % of its potency for a specific period of time (shelf life $t_{90\,\%}$) under defined conditions. It is indispensable that drug manufacturers demonstrate, and routinely ensure that their commercial products maintain identity, quality, and potency and purity throughout shelf life. Many compounds are produced economically on a large scale with purities exceeding 99 % (w/w). Stability studies can be viewed as efforts to achieve the goal of acquiring the knowledge of pathways and the conditions needed to assemble the active pharmaceutical ingredient . The degradation of most pharmaceuticals can be satisfactorily classified as zero order, first order or pseudo first order. Photochemical reactions in pharmaceutical suspensions usually follow zero order kinetics (8-10).

The main variables to be considered in a stability program are temperature, light and moisture. In addition, properties of preservatives, microbial stability and physical characteristics such as color and hardness are a valuable part of many programs. The effect of light on the stability of a drug is usually done in light cabinets with amber bottles. The effect of moisture is studied by varying the humidity, and the effect of temperature is studied in constant temperature cabinets. Stability can always be increased by lowering temperature, but formulations are not usually frozen. Some reactions take place in the solid state than at the same temperature in supercooled solutions (10) . Hydroxyl and ester moieties are some of the organic functional groups capable of

chemical reactions, when pharmaceutical compounds are left in a reactive chemical environment. Environmental factors may be as benign as humidity, elevated temperature, physical contact with excipients , or packaging components in a solid state or solution formulations in which components are packaged. Establishing a pharmaceutical compound stability profile is analogous to establishing its metabolic profile in biological systems (11). Modes of degradation of a pharmaceutical product include (a) physical degradation due to impact vibration, abrasion, and temperature flactuations, such as freezing, thawing or shearing (b) chemical degradation (solvolysis, oxidation etc) and (c) biological (especially microbiological) factors. The most common form of oxidative decomposition that occurs in pharmaceuticals is autoxidation through a free radical chain process. Well structured stability studies are an intergral part of scientific knowledge needed to develop chemical compounds into high quality drug products (11,12).

Effects of dosage forms on stability of drugs

Excipients employed in pharmaceutical formulations have some effect on the stability of drugs. In solution, the effect of excipients and pharmaceutical aids on stability can be more significant than in the solid dosage form. Drugs added to saline or lactated Ringers injection can be unstable since these solutions are not usually buffered (13). Buffers are used to control pH and organic solvents such as alcohol are used to solubilise drugs that affect the dielectric constant of a drug. In addition, preservatives can decompose also. Many parenteral drugs are lyophilised or dry filled into ampoules since they have limited

stability in aqueous solution. Aspirin only occurs in the solid dosage form and does not occur in the liquid dosage form because it is hydrolysed by water. Upon reconstituting drugs with sterile water or other common diluents or when added to intravenous fluids, degradation occurs. Detailed stability on reconstituted preparations must be taken into account to evaluate the effect of line and storage conditions with commonly used vehicles (14).

Effects of admixture vehicles on drug stability

Admixture vehicles such as 0.9% sodium chloride, 0.45% sodium chloride, 5% dextrose, 10 % dextrose and 5 % dextrose with lactated ringer's injections(s) are widely used. Dextrose injection USP is not buffered and its pH range is 3.2 to 6.50. It contains essentially D-glucose monohydrate and water for injection in a flexible plastic container formulated from polyvinyl chloride (PVC). When administered intravenously to a patient, the solution is a source of calories and water (15). Sodium chloride injection USP is sterile and non pyrogenic. The pH range is 4.5 to 7.0 .The injection is a parenteral fluid and electrolyte replenisher, as it contains sodium chloride and water for injection in a flexible plastic PVC container (16).

Effect of containers on the stability of drug

Stability studies in 0.9% sodium chloride injection in various glass containers reveal that the materials in certain glass types and stoppers causes a significant pH increase on

storage due to material release from the stopper upon autoclaving. Interaction between polyethylene containers and drugs has been reported . In some studies glass has been found to be a better container than polyethylene bags for the storage of "cherry laurel" distilled water (17) and interaction between drugs such as phenylephrine and polyethylene containers has been noted to cause loss in stability of the drug , which is significant at ambient temperature (18).

Effect of the environment on drug stability

Natural light and fluorescent light cause discoloration of various pharmaceuticals (19). Coloured or amber plastic containers have been shown to give better protection than white clear containers.When light is prevented by use of such colored containers, the rate of degradation is curtailed. Most drugs are packaged in light resistant containers or with UV absorbers to alleviate the light instability problem (20). Antioxidants such as sodium bisulfite and chelating agents have also been used to preserve drugs (21).

Stability Indicating assay methods

Stability indicating assay methods employ several tools which include thin layer chromatography (TLC) (22), isothermal calorimetry (23), and high performance liquid chromatography (HPLC)(24,25), titrimetric methods, colorimetric, spectrophotometric methods (26), thin layer chromatography (TLC), and gas liquid chromatography (GLC) .

Lately, supercritical fluid chromatography and capillary electrophoresis have attracted attention as separation techniques and have shown stability indicating potential (27-30).

In stability indicating testing, degradation is often measured in a semiquantitative manner and it is essential to assay as accurately and precisely as possible, the amount of intact drug molecule in the presence of its degradation products and formulation ingredients. It is essential to approach the problem in three ways:

(a) by measuring the intact drug molecule: if the method is specific, then separation from degradation products might not be necessary

(b) by measuring specific degradation products, this can only work if there is a suitable stability indicating analytical procedure

(c) by measuring the intact molecule and a specific degradation product

Until recently TLC generally was used as a stability indicating assay in stability studies due to the following reasons: it is cheap, simple, rapid, and all components on the plate can be rendered visible. HPLC is now considered the premier method for stability indicating assays and is more amenable than TLC and GC, because it is rapid, can be automated, is used for nonvolatile, polar and thermally labile compounds and offers more flexibility in the use of chemically bonded stationary phases and there might be no need for sample derivatization as is the case with GC analysis (31-33).

Isocratic analysis by reversed- phase high performance liquid chromatography (RP-HPLC) using a variable wavelength UV detector or Diode Array Detector (DAD) is the prototype stability indicating assay (34). In reversed- phase

chromatography, a C_{18} column with silica bonded to a non polar stationary phase (octadecyl) is usually used to separate the drug sample and its degradants.This type of column contributes to retention of the drug sample, but a more polar mobile phase elutes the sample from the column. Even excipients can contribute to peaks, but they are eluted early by a polar mobile phase. Normal phase chromatography lends itself useful by its polar silica based stationary phase allowing for the resolution of lipophilic drugs that would associate with a non polar stationary phase (35).

Detectors in stability assays

Since many drugs have distinctive chromophores, UV and DAD detectors find much use in chromatography today (36,37). Variable wavelength UV detectors are based on the absorption of UV light by the drug sample or analyte and they are operated at a fixed wavelength and have good sensitivity with a limit of detection (LOD) of 0.01-100ng (38,39). Diode Array Detectors (DAD) are an advanced type of UV detectors with the ability to monitor across the full UV range simultaneously using an array of photodiodes which detect light dispersed by a monochromator over a range of fixed wavelengths. Diode Array Detectors, with their rapid scanning capability can confer rapid development of chromatographic assays. Spectral data from Diode Array Detectors is reinforced with retention time data for aid in peak identification. The LOD for a DAD is 10ng (40). In electrochemical detectors, the drug in the detector cell is converted to its oxidized form (unstable) which is usually converted to the

stable form when it passes through the cell. The instantaneous current generated is proportional to the concentration of the drug in the cell (41).

Pharmacology of zidovudine

Zidovudine is marketed in solid and intravenous dosage forms for oral and intravenous administration. The stability of zidovudine in intravenous solution is crucial because the intravenous form is always diluted prior to administration alone or as an admixture. The drug is found to be stable after dilution for 24 hrs at ambient temperature and 48 hrs if refrigerated at 2 to 8 degrees Celsius. Vials containing zidovudine are stored at 15 to 25 degrees Celsius and protected from fluorescent and natural light (42-43). The stability indicating assays for zidovudine and dobutamine hydrocholoride or ranitidine hydrochloride were developed and validated in our laboratory. These assays involved various conditions, that included forced degradation, pH changes and storage (44) .The stability of dobutamine hydrochloride in peritoneal dialysis solutions at 4, 26, and 37 degrees Celsius has been studied .The drug has been found to be stable in 4.25 % dextrose dialysis solution at these temperatures up to 24 hrs (45). In addition dobutamine hydrochloride has been found to be stable up to 24 hrs in large volume parenteral solutions containing 5 % dextrose injection, 0.9% sodium chloride injection, lactated Ringer's injection, and 4.5% sodium chloride in both glass and polyvinyl chloride containers (46, 47). Since pH can be a factor, the chemical stability of dobutamine hydrochloride is compromised in admixtures containing 5 % sodium bicarbonate as shown by precipitate formation, and formation of extraneous peaks on HPLC analysis.

Dobutamine hydrochloride has been shown to be compatible in admixtures with heparin sodium, lidocaine hydrochloride, procainamide hydrochloride , nitroglycerine , and dopamine (48-50).

Ranitidine occurs as a hydrochloride salt stable in three dosage forms, solid, syrup, and intravenous form. It may be administered through a Y- injection port along with a wide range of medications, which include insulin, antibiotics, antiemetics and antifungals. It has been shown to be stable in 0.9% sodium chloride injection, 5 % dextrose injection, and in PVC bags, glass vials, and plastic syringes (51-53). Stability of ranitidine hydrochloride in total parenteral nutrient solutions (54), intravenous admixtures (55) and amino acids in parenteral solutions (56) has been studied. Storage temperature and time were crucial factors in the storage of ranitidine hydrochloride using a large number of batches frozen or refrigerated until time for use. The other stability variables are pH and humidity. Ranitidine hydrochloride is unstable at low pHs because it is a basic drug (57). Its initial concentration and loss due to evaporation are all factors to be taken into account when the stability studies of this drug are considered. Ranitidine hydrochloride and its metabolites have been analysed by RP-HPLC using fluorescent detection (58), in human serum by RP-HPLC using UV detection , and in rat plasma using RP-HPLC employing a DAD detector (59). There is no literature that indicates that studies of the compatibility of zidovudine with dobutamine hydrochloride and ranitidine hydrochloride in admixture injections stored in PVC bags have been conducted . The purpose of this study was to investigate the stability of zidovudine with dobutamine hydrochloride and

ranitidine hydrochloride admixture injections stored in PVC bags at ambient temperature and 4 degrees Celsius up to 24 hr.

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CHAPTER 2

STABILITY OF ZIDOVUDINE AND DOBUTAMINE HYDROCHLORIDE IN 0.9 % SODIUM CHLORIDE AND 5 % DEXTROSE INJECTIONS AT AMBIENT TEMPERATURE AND 4 CELSIUS STORED IN 50 CC PVC BAGS UP TO 24 HR1.

[1] Musami, P.; J,T, Stewart.; E, W,Taylor. Submitted to *Int J. of Compd.,* 10/20/02

ABSTRACT

The stability of 10 mg/mL zidovudine and 12.5mg/mL dobutamine hydrochloride stored in 0.9% sodium chloride and 5 % dextrose injections in 50cc polyvinyl chloride (PVC) bags stored at 4˚ Celsius and ambient temperature up to 24 hr was studied. Samples were analysed at 0, 4, 8 and 24 hr after preparing the controls and known mixtures, using a reversed phase stability indicating HPLC assay. The drugs mixtures were stable up to 24 hrs as shown by the % remaining drug concentrations above 95 % at the respective sampling times. The pH of the drug solutions remained constant over time. These data support unequivocally the stability of the zidovudine / dobutamine hydrochloride admixture under the storage conditions studied in this investigation.

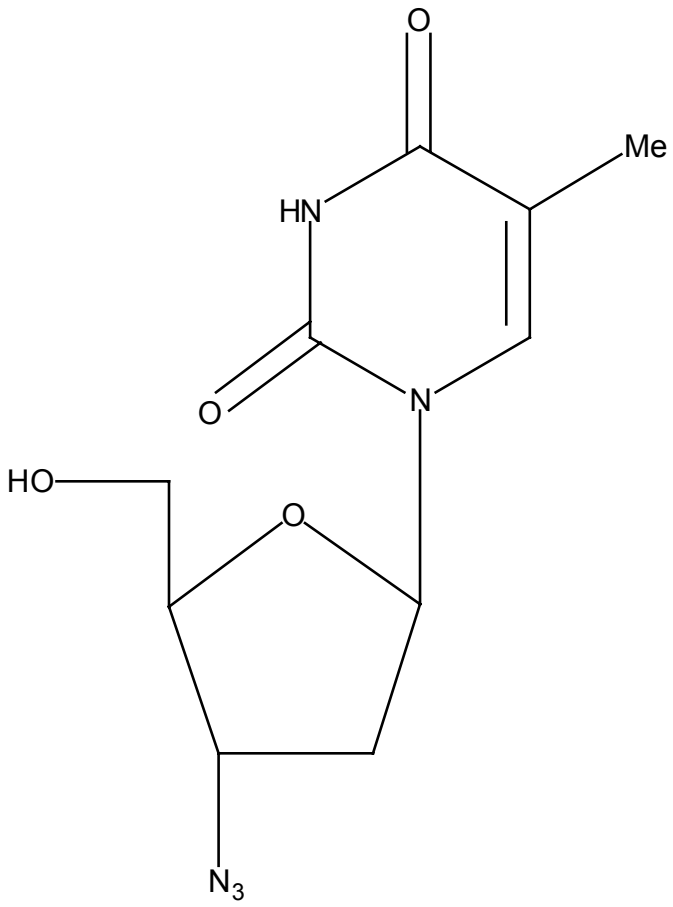
INTRODUCTION

Zidovudine (3´azido-3´-deoxythymidine, a thymidine nucleoside analogue), was approved by the Food and Drug Administration (FDA) in 1987 for the treatment of AIDS (1). It differs from thymidine in having an azido (N_3) group at the 3´ position of the deoxyribose ring (Fig 1.1). It permeates the cell membrane of target cells chiefly by non-facilitated diffusion and the lipophilic azido group imparts the enhanced membrane permeability. It is converted by cellular kinases to zidovudine triphosphate, then competitively inhibits the binding of deoxythymidine triphosphate (dTTP) to reverse transcriptase. In addition, zidovudine acts as an elongation terminator, because the absence of the 3′-OH group prohibits the 5´ to 3´ linkage that is required for the polymerization of the polynucleotide chain. Zidovudine is phosphorylated to zidovudine monophosphate by thymidine kinase. The monophosphate is then phosphorylated to zidovudine diphosphate by a thymidine kinase and then to triphosphate by a nucleoside diphosphokinase. Zidovudine is effective in increasing CD_4 lymphocyte count and decreasing the morbidity and mortality of HIV/AIDS (2,3,4). The solution is usually chemically stable after 24 hr at ambient temperature(23 ± 2 degrees Celsius) and 48 hr if refrigerated at 2- 8 degrees Celsius. Vials containing zidovudine are usually stored at 15 to 25 degrees Celsius. The stability of zidovudine in tablet dosage form has been studied in combinations with other drugs such as ranitidine hydrochloride, dobutamine hydrochloride and certain benzodiazepines using RP-HPLC and UV detection (5), ion-pair liquid chromatography with UV detection (6) and radioimmunoassay (7), and in body matrices such as serum using RP-HPLC (8) with tandem mass spectrometry (9).

Dobutamine hydrochloride (Fig 2.2) is a synthetic catecholamine indicated for short term inotropic support in adults with cardiac decompensation, myocardial infarction, or after cardiac surgery. Dobutamine hydrochloride occurs in one dosage form, as an injection stable at ambient temperature(10,11). Dobutamine hydrochloride acts by stimulating primarily β_1 receptors and to a lesser extent α receptors and β_2 receptors(12).

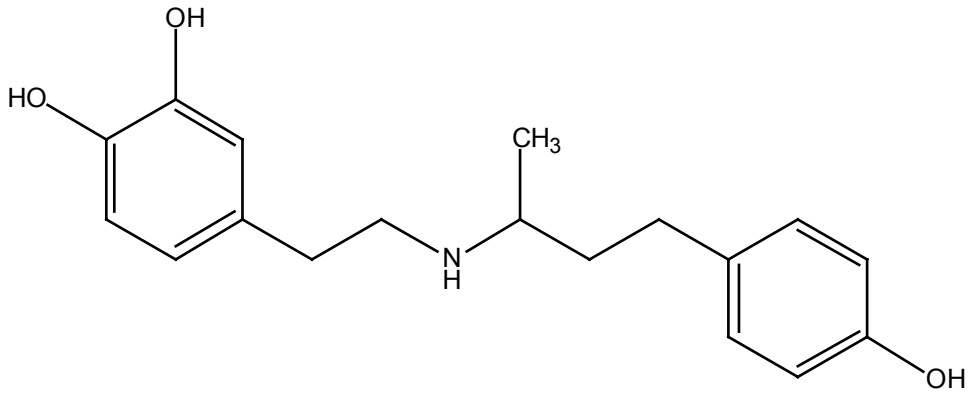
The purpose of this study was to investigate the stability of zidovudine and dobutamine hydrochloride injections when stored as an admixture in PVC bags, at ambient temperature and refrigeration at 4 degrees Celsius up to 24 hr.

FIG 2.1



Chemical structure of zidovudine

Fig 2.2



Chemical structure of dobutamine

EXPERIMENTAL

Chemical and Reagents

All chemicals used were of HPLC grade. Zidovudine and dobutamine hydrochloride reference standards were purchased from USP, (Rockville, MD, USA). Zidovudine infusion vials (10 mg /mL) Lot 1H1585 were purchased from Glaxo Smith Kline , (Research Triangle, NC, USA), and dobutamine hydrochloride injection vials (12.5mg /mL) Lot 891753A were purchased from Abbott Laboratories, (Chicago, IL). 0.9% sodium chloride injection and 5% dextrose injection in 50 cc PVC bags were

purchased from Cardinal Health, (McDonough, GA,USA). Sodium dihydrogen phosphate was purchased from Chemical Research Stores, (UGA Campus). HPLC grade acetonitrile was purchased from Fischer Scientific, (Pittsburgh, PA,USA). Deionised water was HPLC grade, using cartridges from US Filters (Atlanta, GA,USA).

Equipment

The HPLC system consisted of a Beckman Model 110, solvent delivery pump, (Fullerton , CA,USA) a manual Rheodyne injector, (Cotati, CA,USA) and a lambda max UV detector model 481,(Waters, Milford, MA USA). The peak heights were monitored with a Schimadzu, model CR 3A, electronic intergrator, (Columbia, MD, USA.). A 100µL Kloehn syringe(Brea, CA, USA.), a pH meter, built in- house, a C_{16} column that contained an amide hexadecylsilane packing material (4.6mm x 250 mm, 5 µm particle size) (Supelco, Bellefonte, PA, USA.), nylon -66 membrane filters (0.2µm x 47 mm), (Supelco, Bellefonte, PA, USA.), 60 cc syringe ,Tyco Health Systems, Lot 133709, Mansfield, MA USA.). The manual injector was fitted with a 20 µL loop.

Preparation of 25 mM KH_2PO_4 buffer

3.4 grams of KH_2PO_4 salt was weighed on an analytical balance and dissolved in a litre of de-ionised water under constant stirring .The pH of the solution was adjusted to 3.0, using 0.1 M phosphoric acid, after which the buffer solution was mixed with acetonitrile in the ratio of 84:16 (v/v). The mixture was filtered through a 0.20µm nylon filter to

remove particulate matter and microbes.The filtered mobile phase was subsequently sonicated for 10 min to remove dissolved gases.

Chromatographic conditions

The isocratic elution was performed at 1mL/min at ambient temperature. The mobile phase consisted of 16 % acetonitrile and 84 % 25mM sodium phosphate (monobasic) buffer, pH 3.0.

Preparation of reference standard solutions

Two 4 mg quantities of zidovudine USP reference standard were added to 5mL test tubes: 1mL of (a) 0.9 % sodium chloride injection and 1 mL of (b) 5 % dextrose injection was added to give two stock solutions each containing 4 mg/mL. The stock solutions were further diluted, using their respective injection solutions as diluents to final concentration of 2 mg /mL. Two 2 mg quantities of dobutamine hydrochloride USP reference standard were added to 5 mL test tubes: 2 mL of (a) 0.9 % sodium chloride injection and 2 mL of (b) 5 % dextrose injection were added to give two stock solutions each containing 1 mg /mL.

HPLC analysis of the reference standards

A solutions were diluted to give a zidovudine concentration of 25µg/mL and a dobutamine hydrochloride concentration of 12.5 µg/mL. Injections of 100 µL were made of the diluted solutions in triplicate into the HPLC system to calculate the mean response factors (MRFs) for zidovudine and dobutamine hydrochloride.

Preparation of controls in 50 cc PVC bags

A pooled sample of 10 mL (10 mg/mL) of zidovudine injection was pipetted into each of the four 50 cc amber volumetric flasks to avoid photodegradation and (a) 40 mL of 0.9 % sodium chloride injection and (b) 40 mL of 5 % dextrose injection were added to give a concentration of 2 mg /mL in each control. The mixtures were vortex mixed to ensure complete dissolution of the drug. These were labeled as controls and decanted into each of the four labelled control 50cc PVC bags. A pooled sample of 4 mL (12.5mg /mL) of dobutamine were aliquoted into each of the four amber 50cc volumetric flasks and brought to the mark using 46 mL of (a) 0.9 % sodium chloride injection and (b) 46 mL of 5 % dextrose injection to give a concentration of 1 mg /mL in each control. The control solutions were vortex mixed decanted into each of the four labelled control 50cc PVC bags. Half of the control bags were stored at ambient temperature (23 ± 2°C) and the other half refrigerated at 4 ± 1°C .

Preparation of the admixtures in 50 cc PVC bags

10 mL (10 mg /mL) of zidovudine were pipetted into each of the six amber 50cc volumetric flasks, followed by 4 mL (12.5mg /mL) of dobutamine and brought to the mark using 36 mL of (a) 0.9% sodium chloride injection and (b) 36 mL of 5 % dextrose injection, giving concentrations of 2mg /mL for zidovudine and 1 mg /mL for dobutamine. The admixtures were vortex mixed and decanted into each of the six 50cc PVC bags using a 60 cc syringe fitted with a 20 ½ gauge needle. Half of the admixture bags were stored at ambient temperature (23 ± 2 °C)and the other half refrigerated at 4 ± 1° C.

Preparation of Assay solutions

5 mL of each of the 50 cc PVC bags contents stored at ambient temperature and 4 °C were removed at 0, 4, 8, and 24 hr, visually examined for color change, turbidity, and occurrence of precipitation or gas formation, the pH recorded, and a 1 in 20 dilution made with the respective injection solutions, to give final concentrations for zidovudine of 20 µg /mL and 54 µg /mL for dobutamine hydrochloride.

HPLC analysis

100 µL of the diluted controls and admixtures were injected in triplicate on the HPLC system.The system was operated isocratically at a flow rate of 1 mL/ min, using a C_{16}

amide column at ambient temperature, a UV detector operated at 280nm, and acetonitrile-phosphate buffer (pH 3.0) as mobile phase.

Calculation of Medication Content

Peak heights from the chromatograms were used to calculate the mean response factor (MRF) for each drug standard. The appropriate MRF and the peak height of the analyte in the PVC bag and standard samples were used to calculate the drug concentration in each analytical sample. The following procedure was used to calculate the drug concentration in each analytical sample:

1- Response Factor (RF) = Drug standard (mg/mL)/ Drug peak height of standard.

2- Calculate the Mean Response Factor (MRF), which is based on three replicates of the RF of the standard.

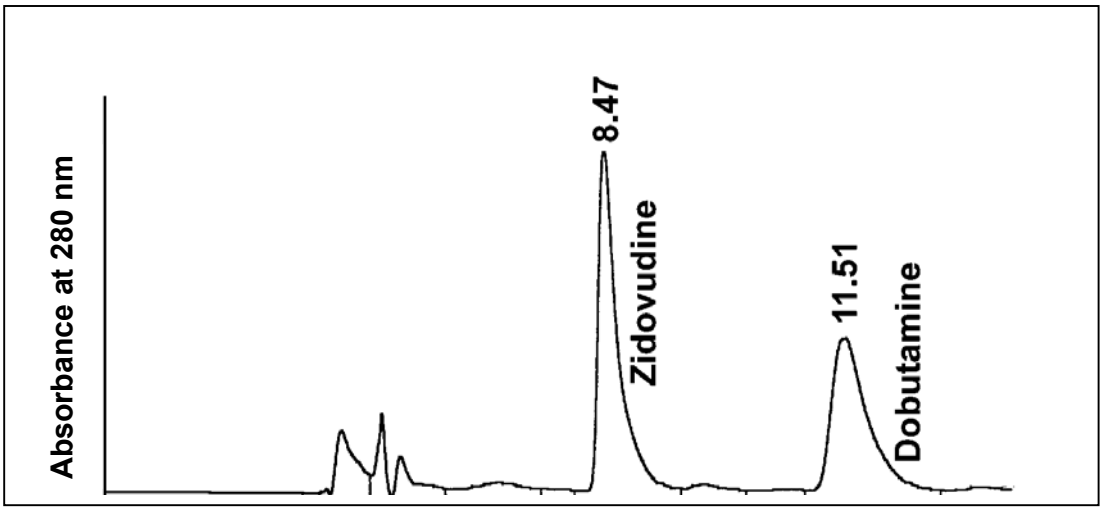
3- Drug concentration (mg/mL) = MRF x drug peak height of sample

Results

The HPLC retention time of zidovudine was approximately 8.47 min and that of dobutamine hydrochloride was 11.51 min. All the experiments confirmed that neither the infusion fluid nor the phenol preservative in the zidovudine interfered with the accurate quantitation of zidovudine and dobutamine. It shows that the admixture is compatible as there was no color change or precipitation. The zidovudine and dobutamine

hydrochloride admixture, just like their controls, did not lose stability, as their ($t_{90\%}$) remained above 95 % within 24 hr of the study period. This study indicated that since dobutamine hydrochloride is administered in emergency situations, normally within eight hours, it still remains potent for an even longer time(24 hr). In conclusion, zidovudine and dobutamine hydrochloride admixtures are stable for up to 24 hr at ambient temperature and 4° C in PVC bags.

Fig 2.3



Typical chromatogram of zidovudine and dobutamine on a C_{16} amide column

Table 2. 1

Mean pH data for dobutamine hydrochloride and zidovudine controls and mixtures in 0.9 % sodium chloride stored in 50 cc PVC bags at ambient temperature(23 ± 2degrees Celsius) up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	6.36	6.20	6.66	6.70
2[b]	6.76	6.60	6.55	6.34
3[c]	6.20	5.29	6.40	6.02
4[c]	6.80	6.90	6.85	6.30
5[c]	6.65	6.70	6.20	6.11

a. 2mg/ml zidovudine control, in 50 cc PVC bag

b. 1mg/ml dobutamine control, in 50 cc PVC bag

c. 2mg/ml zidovudine and 1mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2.2

Mean pH data for dobutamine and zidovudine controls and mixtures in 5 % dextrose injection at ambient temperature(23 ± 2 degrees Celsius) stored in 50 cc PVC bags up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	6.80	6.20	6.90	6.70
2[b]	6.90	6.76	6.85	6.01
3[c]	6.20	6.50	6.09	6.10
4[c]	6.30	6.10	6.89	6.20
5[c]	6.30	6.50	6.90	6.28

a. 2mg/mL zidovudine control in 50cc PVC bag

b. 1mg/mL dobutamine control in 50cc PVC bag

c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2. 3
Mean pH data for dobutamine and zidovudine controls and mixtures in 0.9% sodium chloride injection stored in 50cc PVC bags at 4 degrees Celsius up to 24 hr

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	6.09	6.15	6.20	6.70
2[b]	6.80	6.65	6.55	6.03
3[c]	6.35	6.40	6.20	6.18
4[c]	6.45	6.60	6.34	6.06
5[c]	6.01	6.16	6.75	6.42

a. 2mg/ml zidovudine control in 50cc PVC bag

b. 1mg/mL dobutamine control in 50cc PVC bag

c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2. 4
Mean pH data for dobutamine and zidovudine controls and mixtures in 5 % dextrose injection in 50cc PVC bags at 4 degrees Celsius up to 24 hr .

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	6.10	6.15	6.38	6.40
2[b]	6.76	6.36	6.06	6.45
3[c]	6.65	6.80	6.43	6.50
4[c]	6.10	6.46	6.74	6.24
5[c]	6.09	6.76	6.20	6.60

a. 2mg/mL zidovudine control in 50cc PVC bag

b. 1mg/mL dobutamine control in 50cc PVC bag

c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50cc PVC bag

Table 2.5

Mean remaining concentration (mg/mL) of dobutamine and zidovudine controls and mixtures in 0.9 % sodium chloride injection in 50cc PVC bags at ambient temperature (23 ± 2 degrees Celsius) up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	2.00± 0.02	1.99± 0.01	2.01 ± 0.01	1.96 ± 0.01
2[b]	1.01± 0.01	1.01± 0.01	0.99± 0.00	0.98 ± 0.00
3[c]	2.01± 0.01	1.97 ± 0.01	1.97± 0.01	1.96 ± 0.01
	0.98 ± 0.00	0.98 ± 0.00	0.97± 0.00	0.96± 0.00
4[c]	2.00± 0.01	2.00± 0.01	1.97 ± 0.02	1.96± 0.01
	1.00± 0.01	1.00 ± 0.01	0.98± 0.00	0.98± 0.00
5[c]	2.00 ± 0.00	2.02± 0.01	1.99± 0.01	1.95± 0.01
	1.02 ± 0.01	1.03± 0.00	1.00 ± 0.01	0.98± 0.00

a. 2mg/mL zidovudine control in 0.9% sodium chloride in 50 cc PVC bag

b. 1 mg/mL dobutamine control in 0.9% sodium chloride in 50 cc PVC bag

c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2.6

Mean remaining concentration (mg/mL) for dobutamine and zidovudine controls and mixtures in 5 % dextrose injection in 50 cc PVC bags stored at ambient temperature (23 ± 2 degrees Celsius) up to 24 hr

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	1.99± 0.01	1.99± 0.01	2.01± 0.02	1.95 ± 0.00
2[b]	1.04± 0.01	1.05± 0.00	1.02± 0.01	0.99 ± 0.00
3[c]	2.02 ± 0.01	1.97± 0.02	1.97± 0.01	1.92 ± 0.01
	1.02± 0.01	1.04 ± 0.01	1.00± 0.00	1.00± 0.00
4[c]	2.03± 0.01	1.99± 0.01	2.00± 0.01	1.95 ± 0.00
	1.00 ± 0.00	0.98± 0.00	1.00 ± 0.00	0.99 ± 0.00
5[c]	2.01 ± 0.01	1.99± 0.00	1.94± 0.02	1.96± 0.00
	1.03 ± 0.01	1.02± 0.01	1.02± 0.01	0.99± 0.01

a. 2mg/mL zidovudine in 5 % dextrose in 50 cc PVC bag

b. 1mg /mL dobutamine in 5 % dextrose in 50 cc PVC bag

c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2.7

Mean remaining concentration (mg/mL) of dobutamine and zidovudine controls and mixtures in 0.9% sodium chloride injection in 50cc PVC bags stored at 4 degrees Celsius up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	1.98 ± 0.01	2.00 ± 0.00	2.00 ± 0.01	1.96 ± 0.01
2[b]	1.10 ± 0.00	1.10 ± 0.00	1.10 ± 0.00	1.09 ± 0.01
3[c]	2.04 ± 0.00	2.04 ± 0.01	2.00 ± 0.01	1.96 ± 0.01
	1.09 ± 0.01	1.08 ± 0.01	1.10 ± 0.00	1.10 ± 0.00
4[c]	2.05 ± 0.00	2.06 ± 0.02	2.05 ± 0.02	2.05 ± 0.00
	1.10 ± 0.00	1.07± 0.05	1.07 ± 0.05	1.10 ± 0.00
5[c]	2.03 ± 0.00	2.07± 0.01	2.03 ± 0.00	1.99 ± 0.00
	1.09 ± 0.01	1.07 ± 0.05	1.10 ± 0.00	1.08 ± 0.01

a. 2mg/mL zidovudine in 0.9 % sodium chloride in 50 cc PVC bag

b. 1mg/mL dobutamine in 0.9% sodium chloride in 50 cc PVC bag

c. 2mg/mL zidovudine and 1 mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2.8

Mean concentration remaining (mg/mL) for dobutamine and zidovudine controls and mixtures in 5 % dextrose injection in 50cc PVC bags at 4 degrees Celsius up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	2.00 ± 0.01	2.02 ± 0.01	2.04 ± 0.00	2.00 ± 0.01
2[b]	1.05 ± 0.01	1.07 ± 0.01	1.05 ± 0.03	1.02 ± 0.01
3[c]	1.99 ± 0.00	2.02 ± 0.00	1.97 ± 0.02	1.96 ± 0.02
	1.04 ± 0.04	1.02 ± 0.00	1.01 ± 0.01	1.00 ± 0.01
4[c]	1.98 ± 0.00	2.00 ± 0.00	1.93 ± 0.00	1.95 ± 0.01
	0.99 ± 0.00	1.01 ± 0.01	0.98 ± 0.00	0.99 ± 0.01
5[c]	1.99 ± 0.01	2.01 ± 0.01	1.96 ± 0.01	1.97 ± 0.00
	1.02 ± 0.01	1.03 ± 0.00	1.03 ± 0.04	1.01± 0.01

a. 2mg/mL zidovudine in 5 % dextrose in 50cc PVC bag
b. 1mg/mL dobutamine in 5 % dextrose in 50 cc PVC bag
c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2.9

Mean percent remaining of dobutamine and zidovudine controls and mixtures in 0.9% sodium chloride injection in 50cc PVC bags at ambient temperature (23 ± 2degrees Celsius) up to 24 hr

Bag	0 hr	4 hr	8 hr	24 hr
1a	100.00 ± 0.00	99.43 ± 0.61	100.17 ± 0.60	97.77 ± 0.45
2b	100.00 ± 0.00	99.43 ± 3.09	97.13 ± 0.41	96.07 ± 0.54
3c	100.00 ± 0.00	98.27 ± 0.94	97.63 ± 0.41	97.1 ± 0.37
	100.00 ± 0.00	100.33 ± 0.63	98.63 ± 0.21	97.67 ± 0.12
4c	100.00 ±0.00	101.40 ± 0.43	98.23 ± 0.81	97.73 ± 0.31
	100.00 ± 0.00	100.90 ± 0.83	98.60 ± 0.37	98.33 ± 0.47
5c	100.00 ±0.00	99.63 ± 0.39	97.93 ± 0.40	96.30 ± 0.50
	100.00 ± 0.00	101.03 ± 0.34	97.57 ± 0.69	95.67 ± 0.46

a. 2mg/mL zidovudine control in 0.9% sodium chloride in 50cc PVC bag
b. 1mg/mL dobutamine control in 0.9% sodium chloride in 50 cc PVC bag
c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2.10

Mean percent remaining for dobutamine and zidovudine controls and mixtures in 5 % dextrose injection in 50cc PVC bags ambient temperature (23 ± 2) degrees Celsius up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	100.00 ± 0.00	99.60 ± 0.48	100.60 ± 0.78	97.80 ± 0.42
2[b]	100.00 ± 0.00	101.40 ± 0.45	98.57 ± 0.41	95.70 ± 0.57
3[c]	100.00 ± 0.00	97.70 ± 0.73	97.78 ± 0.41	95.20 ± 0.64
	100.00 ± 0.00	97.70 ± 0.75	98.80 ± 0.43	98.03 ± 0.37
4[c]	100.00 ± 0.00	98.10 ± 0.70	98.33 ± 0.61	96.27 ± 0.33
	100.00 ± 0.00	97.80 ± 0.30	100.23 ± 0.77	98.40 ± 0.70
5[c]	100.00 ± 0.00	98.37 ± 0.38	95.80 ± 0.90	96.63 ± 0.24
	100.00 ± 0.00	98.87 ± 0.80	98.83 ± 0.93	96.20 ± 0.70

a. 2mg/mL zidovudine control in 5 % dextrose ,in 50 cc PVC bag

b. 1mg/mL dobutamine control in 5 % dextrose, in 50 cc PVC bag

c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50 cc PVC bag

Table 2.11

Mean percent remaining of dobutamine and zidovudine controls and mixtures in 0.9%sodium chloride injection in 50cc PVC bags stored at 4 degrees Celsius up to 24 hr

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	100.00 ± 0.00	100.90 ± 0.24	100.90 ± 0.59	99.30 ± 0.24
2[b]	100.00 ± 0.00	99.67 ± 0.74	100.00 ± 0.78	97.63 ± 0.26
3[c]	100.00 ± 0.00	99.77 ± 0.48	97.73 ± 0.41	95.67 ± 0.53
	100.00 ± 0.00	98.93 ± 0.68	101.30 ± 0.65	99.67 ± 0.42
4[c]	100.00 ± 0.00	100.40 ± 0.96	100.23 ± 0.64	99.70 ± 0.36
	100.00 ± 0.00	99.93 ± 0.24	98.20 ± 0.14	98.53 ± 0.52
5[c]	100.00 ± 0.00	102.10±0.30	101.63 ± 0.45	99.77 ± 0.24
	100.00 ± 0.00	102.30 ± 0.50	102.20 ± 0.43	99.33 ± 0.56

a. 2mg/mL zidovudine control in 0.9% sodium chloride in 50cc PVC bag

b. 1mg/mL dobutamine control in 0.9% sodium chloride in 50 cc PVC bag

c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50cc PVC bag

Table 2.12

Mean percent remaining of dobutamine and zidovudine controls and mixtures in 5 % dextrose injection in 50cc PVC bags at 4 degrees Celsius stored up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	100.00 ± 0.00	100.90 ± 0.67	102.03 ± 0.23	99.97 ± 0.52
2[b]	100.00 ± 0.00	102.17 ± 0.54	98.00 ± 0.51	97.10 ± 0.94
3[c]	100.00 ± 0.00	101.63 ± 0.33	99.67 ± 0.85	98.57 ± 0.75
	100.00 ± 0.00	101.23 ± 0.40	99.67 ± 0.93	98.53 ± 0.53
4[c]	100.00 ± 0.00	101.07 ± 0.50	97.63 ± 0.19	98.90 ± 0.50
	100.00 ± 0.00	102.37 ± 0.94	98.30 ± 0.08	99.30 ± 0.54
5[c]	100.00 ± 0.00	101.03 ± 0.54	98.67 ± 0.40	98.90 ± 0.22
	100.00 ± 0.00	101.30 ± 0.50	98.20 ± 0.00	99.40 ± 1.07

a. 2mg/mL zidovudine control in 5 % dextrose in 50 cc PVC bag

b. 1mg/mL dobutamine control in 5 % dextrose in 50cc PVC bag

c. 2mg/mL zidovudine and 1mg/mL dobutamine in a mixture in 50cc PVC bag

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CHAPTER 3

STABILITY OF ZIDOVUDINE AND RANITIDINE HYDROCHLORIDE INJECTIONS IN 0.9 % SODIUM CHLORIDE AND 5 % DEXTROSE INJECTIONS AT AMBIENT TEMPERATURE (23 ± 2 ºC) AND 4 C STORED IN 50 CC PVC BAGS UP TO 24 HR.[1]

[1] Musami, P.; J, T, Stewart.; E, W, Taylor. Submitted to *Int. J. Pharm. Compd.*, 10/20/02

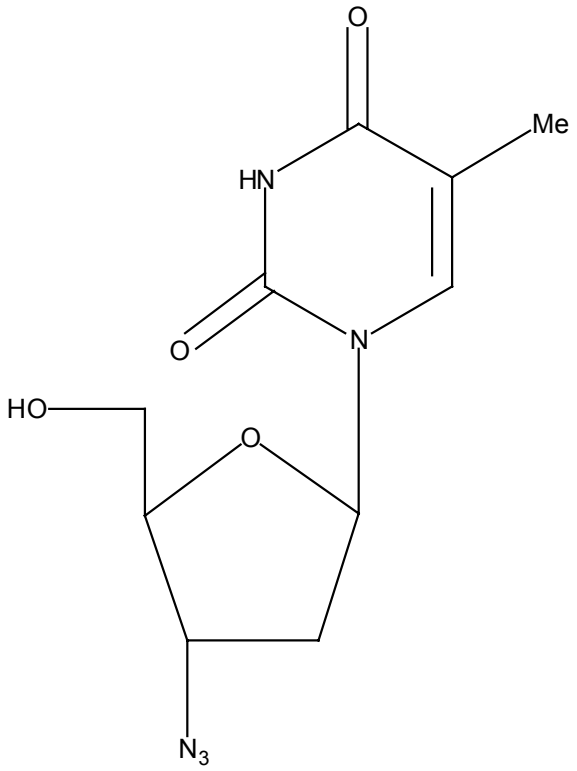
ABSTRACT

The stability of 10mg/mL zidovudine and 25mg/mL ranitidine hydrochloride injections stored in 0.9% sodium chloride and 5 % dextrose injections in 50cc polyvinyl chloride (PVC) bags stored at 4 ºCelsius and ambient temperature up to 24 hr was studied. The samples were analysed at 0, 4, 8 and 24 hr after preparing the controls and admixtures, using a reversed phase stability indicating HPLC assay. The drugs were stable up to 24 hr as shown by the % remaining drug concentrations at the respective sampling times. The pH of the drug solutions remained constant . These data support unequivocally the stability of the zidovudine / ranitidine hydrochloride admixture under the storage conditions used in this investigation.

INTRODUCTION

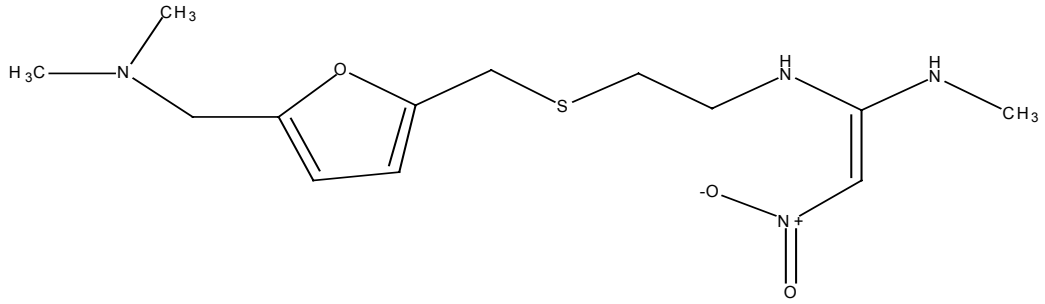
Ranitidine hydrochloride (Fig 3.1) is an H_2 receptor agonist, widely used for the treatment of gastric and duodenal ulcers (1), Zollinger Ellison Syndrome (2), reflux esophagitis (3), dyspepsia and other hypersecretory conditions (5). Ranitidine hydrochloride is a competiive inhibitor of histamine at the H_2 receptors, including receptors in the gastric cells. It is given to critically ill patients who are prone to develop stress ulcers in a "piggy backed" fashion via a primary intravenous line, usually in combination with other drugs (6,7). The stability of zidovudine (Fig. 3.1) and ranitidine hydrochloride (Fig 3.2) as an admixture in PVC bags has never been studied. Therefore the objective of this study was to demonstrate the stability of the two drugs in 0.9 % sodium chloride injection and 5 % dextrose injection at ambient temperature and 4º C when stored in PVC bags up to 24 hr.

Fig 3.1



Chemical structure of zidovudine

Fig 3.2



Chemical structure of ranitidine

EXPERIMENTAL

Chemical and Reagents

All chemicals used were of HPLC grade. Zidovudine and ranitidine hydrochloride reference standards were purchased from USP (Rockville, MD, USA). Zidovudine infusion vials (10mg /mL) Lot 1H1585 were purchased from Glaxo Smith Kline (Research Triangle, NC, USA) and ranitidine hydrochloride injection vials (25mg /mL) Lot 891753A were purchased from Abbott Laboratories, Chicago, Il, USA. 0.9% sodium chloride injection and 5% dextrose injection PVC bags were purchased from Cardinal Health, McDonough, GA, USA.Sodium dihydrogen phosphate was purchased from

Chemical Research Stores, UGA campus. HPLC grade acetonitrile was purchased from Fischer Scientific (Pittsburgh, PA, USA). Deionised water was HPLC grade, using cartridges provided by US Filters (Atlanta, GA,USA).

Equipment

The HPLC system consisted of a Beckman Model 110 solvent delivery pump, (Fullerton, CA, USA) a manual Rheodyne injector,(Cotati, CA, USA.) lambda max UV detector model 481,Waters,(Milford, MO, USA.). The peak heights were monitored with a Schimadzu electronic intergrator (Columbia, MA, USA), a 100µL Kloehn syringe, (Brea, CA, USA.) an in house built pH meter, a C_{16} column that contained an amide hexadecylsilane packing material (4.6mm x 250 mm, 5 µm particle size), (Supelco, Bellefonte, PA, USA). Nylon -66 membrane filters 0.2µm x 47 mm, (Supelco, Bellefonte, PA, USA). The manual injector was fitted with a 20 µL loop, 60 cc syringe, Tyco Health System Lot 133709, (Mansfield, MA, USA).

Preparation of 25 mM KH_2PO_4 buffer

3.4 grams of KH_2PO_4 salt was weighed on an analytical balance and dissolved in a litre of de-ionised water under constant stirring .The pH of the solution was adjusted to 3.0, using 0.1 M phosphoric acid, after which the buffer solution was mixed with acetonitrile in the ratio of 88:12 (v / v). The mixture was filtered through 0.20µm nylon filter to remove particulate matter and microbes.The filtered mobile phase was subsequently sonicated for 10 min to remove dissolved gases.

Chromatographic conditions

The isocratic elution was performed at 1mL/min at ambient temperature.The mobile phase consisted of 12 % acetonitrile and 88 % 25 mM sodium phosphate monobasic buffer pH 3.0.

Preparation of reference standard solutions

Two 4 mg quantities of zidovudine USP reference standard were added to two 5mL test tubes: 1mL of (a) 0.9% sodium chloride injection and 1 mL of (b) 5 % dextrose injection was added to give stock solutions containing 4 mg/mL. The stock solutions were further diluted, using their respective injection solutions as diluents to 2 mg/mL.

Two 1 mg /mL quantities of ranitidine hydrochloride USP reference standard were added to 5 mL test tubes: 1 mL of (a) 0.9% sodium chloride injection and 1 mL of (b) 5 % dextrose injection were added to give two stock solutions each with a concentration of 1 mg /mL. Each of the stock solutions was further diluted to give a 0.5 mg /mL solution.

HPLC analysis of the reference standards

The zidovudine stock solution (2 mg /mL) was diluted to a final concentration of 20 µg/mL and ranitidine hydrochloride stock solution (0.5 mg/ mL) was diluted to a final concentration of 12.5 µg /ml. 100µL of the diluted solutions were injected in triplicate onto the HPLC and used to calculate the Mean Response Factors (MRFs) of the reference standards.

Preparation of controls in 50cc PVC bags

A pooled sample of 10mLs (10 mg/mL) of zidovudine injection was pipetted into each of the four 50cc amber volumetric flasks to avoid photodegradation and 40 mL of (a) 0.9% sodium chloride injection (b) 40 mL of 5 % dextrose injection, were added to give a nominal concentration of 2mg /mL in each control. The mixtures were vortex mixed to ensure complete dissolution of the drug. These were labeled as controls and decanted into each of the four control 50cc PVC bags.

A pooled sample of 1 mL (25mg /mL) of ranitidine were aliquoted into each of the four amber 50cc volumetric flasks and brought to the mark using 49 mLs of (a) 0.9 % sodium chloride injection and 49 mLs of (b) 5 % dextrose injection to give a concentration of 0.5mg /mL in each control. The control solutions were vortex mixed and decanted into each of the four control 50cc PVC bags. Half of the control bags were stored at ambient temperature (23 ± 2°C) and the other half refrigerated at 4 ±1°C .

Preparation of the admixtures in 50cc PVC bags

10mL (10mg /mL) of zidovudine were pipetted into each of the six amber 50cc volumetric flasks, followed by 1 mL (25mg /mL) of ranitidine and brought to volume using 39 mL of (a) 0.9% sodium chloride injection and 39 mL of (b) 5 % dextrose injection, giving concentrations of 2mg /mL for zidovudine and 0.5 mg /mL for ranitidine. The admixtures were vortex mixed and decanted into each of the six 50cc PVC bags using a 60 cc syringe fitted with a 20 ½ gauge needle. Half of the admixture bags were stored at ambient temperature (23 ± 2 °C)and the other half refrigerated at 4 ± 1°C.

Preparation of assay solutions

5 mL from each of the 50cc PVC bags stored at ambient temperature and 4 ºC was removed at , 0, 4, 8, and 24 hr, visually examined for color change, turbidity, and

occurrence of precipitation or gas formation, the pH recorded, a 1 in 20 dilution made with the respective injection solutions, to give final concentrations for zidovudine of 20 µg/mL and 12.5 µg /mL for ranitidine hydrochloride.

HPLC analysis

100 µL of the diluted controls and admixtures were injected in triplicate on the HPLC system.The system was operated isocratically at a flow rate of 1 mL/ min using a C_{16} amide column at ambient temperature, the UV detector operated at 265 nm, using acetonitrile-phosphate buffer (pH 3.0) as mobile phase.

Calculation of Medication Content

Peak heights from the chromatograms were used to calculate the mean response factors (MRFs) for each drug standard. The appropriate MRF and the peak height of the analyte in the PVC bag and standard samples were used to calculate the drug conentration in each analytical sample. The following procedure was used to calculate the drug concentration in each analytical sample:

1 - Response Factor (RF) = Drug standard (mg/mL)/ Drug peak height of standard.

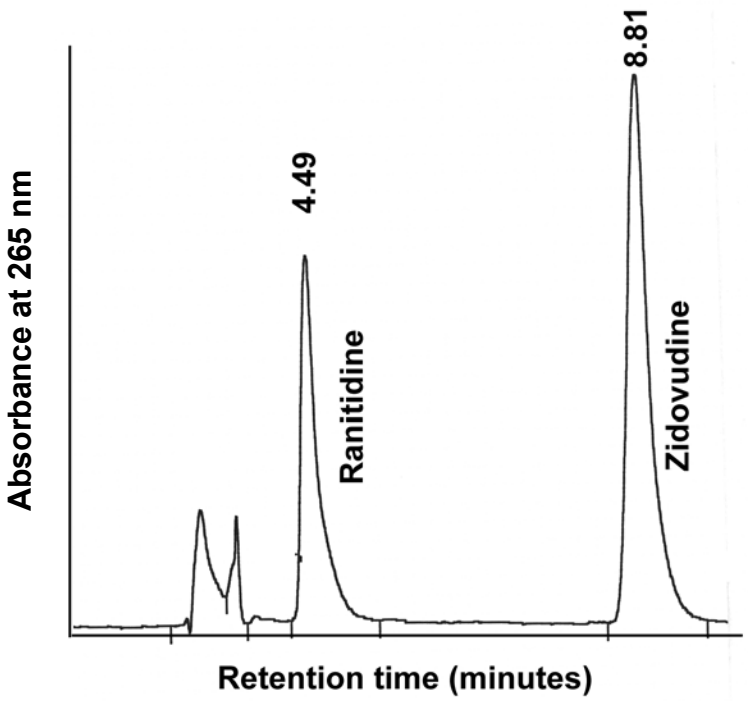
2- Calculate the mean response factor (MRF), based on three replicates of RF of each standard.

3- Drug concentration (mg/mL) = MRF x drug peak height of sample

Results

The HPLC retention time of zidovudine was approximately 8.81 min and that of ranitidine was 4.49 min. All the experiments confirmed that neither the infusion fluid, nor the phenol preservative in the zidovudine, interfered with the accurate quantitation of zidovudine and ranitidine. It showed that the admixture was compatible as there was no color change or precipitation. The zidovudine and ranitidine admixtures, just like their controls, did not lose stability, as their ($t_{90\%}$) remained above 90% up to 24 hr of the study period. Therefore zidovudine and ranitidine can be administered as an admixture and remained stable up to 24 hr .

Fig 3.3



Typical chromatogram of ranitidine and zidovudine on a C_{16} amide column

Table 3.1

Mean pH data for zidovudine and ranitidine controls and mixtures in 0.9% sodium chloride injection in 50 cc PVC bags stored at ambient temperature (23 ± 2 degrees Celsius) up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1a	5.90	5.66	5.33	5.30
2b	5.46	5.58	5.45	5.44
3c	5.98	5.72	5.45	5.55
4c	5.81	5.48	5.33	5.45
5c	5.93	5.23	5.81	5.07

a. 2mg/ml zidovudine control in 50cc PVC bag

b. 0.5mg/ml ranitidine control in 50cc PVC bag

c. 2mg/ml zidovudine and 0.5mg ranitidine in a mixture in 50cc PVC bag

Table 3. 2

Mean pH data for zidovudine and ranitidine controls and mixtures in 5 % dextrose injection in 50cc PVC bags at ambient temperature (23 ± 2 degrees Celsius) stored up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1a	5.10	5.16	5.09	5.80
2b	5.70	5.64	5.50	5.96
3c	5.20	5.73	5.98	5.92
4c	5.45	5.68	5.77	5.71
5c	5.33	5.98	5.67	5.07

a. 2mg/ml zidovudine control in 50cc PVC bag

b. 0.5mg/ml ranitidine control in 50cc PVC bag

c. 2mg/ml zidovudine and 0.5mg/ml ranitidine in a mixture in 50cc PVC bag

Table 3..3

Mean pH data for zidovudine and ranitidine controls and mixtures in 0.9% sodium chloride injection in 50cc PVC bags stored at 4 degrees Celsius up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1a	5.06	5.21	5.72	5.63
2b	5.39	5.78	5.01	5.40
3c	5.05	5.59	5.68	5.40
4c	5.10	5.92	5.70	5.35
5c	5.38	5.60	5.85	5.90

a. 2mg/ml zidovudine control in 50cc PVC bag

b. 0.5mg/ml ranitidine control in 50cc PVC bag

c. 2mg/ml zidovudine and 0.5mg/ml ranitidine in a mixture in 50 cc PVC bag

Table 3. 4

Mean pH values for zidovudine and ranitidine controls and mixtures in 5% dextrose injection in 50cc PVC bags stored at 4 degrees Celsius up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1a	5.46	5.32	5.45	5.34
2b	5.86	5.98	5.76	5.50
3c	5.78	5.55	5.49	5.50
4c	5.90	5.76	5.68	5.47
5c	5.17	5.07	5.34	5.56

a. 2mg/ml zidovudine control in 50cc PVC bag

b. 0.5 mg/ml ranitidine control in 50 cc PVC bag

c. 2mg/ml zidovudine and 0.5mg/ml ranitidine in a mixture in 50 cc PVC bag

Table 3.5

Mean remaining concentration (mg/mL) of zidovudine and ranitidine controls and mixture in 0.9% sodium chloride injection in 50 cc PVC bags at ambient temperature (23 ± 2 degrees Celsius) up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	2.09 ± 0.01	2.10 ± 0.0	2.11 ± 0.01	2.11 ± 0.01
2[b]	0.49 ± 0.00	0.50 ± 0.01	0.50 ± 0.01	0.51 ± 0.01
3[c]	2.08 ± 0.01	2.09 ± 0.01	2.09 ± 0.01	2.08 ± 0.01
	0.50 ± 0.0	0.50 ± 0.00	0.50 ± 0.01	0.50 ± 0.01
4[c]	2.08 ± 0.01	2.08 ± 0.01	2.10 ± 0.01	2.07 ± 0.03
	0.49 ± 0.01	0.50 ± 0.01	0.50 ± 0.01	0.51 ± 0.01
5[c]	2.08 ± 0.02	2. 09± 0.0	2.09 ± 0.01	2.08 ± 0.02
	0.49 ± 0.01	0.49 ± 0.01	0.50 ± 0.01	0.50 ± 0.01

a. 2mg/mL zidovudine control in 50cc PVC bag

b. 0.5mg/mL ranitidine control in 50 cc PVC bag

c. 2mg/mL zidovudine and 0.5mg/mL ranitidine in a mixture in 50 cc PVC bag

Table 3.6

Mean concentration (mg/mL) remaining of zidovudine and ranitidine controls and mixture in 5 % dextrose injection at ambient temperature (23±2 degrees Celsius) up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	2.20 ± 0.01	2.20 ± 0.01	2.10 ± 0.01	2.07 ± 0.02
2[b]	0.49 ± 0.01	0.49 ± 0.01	0.49 ± 0.0	0.49 ± 0.01
3[c]	2.20± 0.01	2.10 ± 0.02	2.04 ± 0.01	2.02 ± 0.01
	0.49 ± 0.00	0.46 ± 0.01	0.45 ± 0.01	0.44 ± 0.0
4[c]	2.15 ± 0.01	2.13 ± 0.01	2.04 ± 0.02	2.02 ± 0.01
	0.49 ± 0.01	0.46 ± 0.01	0.45 ± 0.01	0.45 ± 0.0
5[c]	2.20 ± 0.09	2.10 ± 0.02	2.04 ± 0.01	2.04 ± 0.01
	0.49 ± 0.01	0.49 ± 0.01	0.45 ± 0.01	0.43 ± 0.01

a. 2mg/mL zidovudine control, in 50 cc PVC bag

b. 0.5 mg/mL ranitidine control, in 50 cc PVC bag

c. 2 mg/mL zidovudine and 0.5mg/mL ranitidine in a mixture in 50 cc PVC bag

Table 3. 7

Mean concentration (mg/mL) remaining of zidovudine and ranitidine controls and mixtures in 0.9% sodium chloride injection in 50cc PVC stored at 4 degrees Celsius up to 24 hr.

Bag	0 hrs	4 hrs	8 hrs	24 hrs
1[a]	2.02 ± 0.01	2.0± 0.01	2.0 ± 0.02	2.01 ± 0.02
2[b]	0.49 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.50 ± 0.01
3[c]	1.98 ± 0.01	2.02 ± 0.01	2.01 ± 0.01	1.99 ± 0.02
	0.50 ± 0.01	0.50 ± 0.0	0.49 ± 0.01	0.49 ± 0.01
4[c]	2.0 ± 0.02	2.02 ± 0.01	2.0 ± 0.02	1.98 ± 0.01
	0.50 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.49 ± 0.01
5[c]	1.99 ± 0.01	2.02 ± 0.01	2.04 ± 0.01	1.98 ± 0.01
	0.51 ± 0.0	0.51± 0.01	0.49 ± 0.01	0.49 ± 0.01

a. 2mg/mL zidovudine control in 50 cc PVC bag

b. 0.5mg/mL ranitidine control in 50 cc PVC bag

c. 2mg/mL zidovudine and 0.5mg/mL ranitidine control in a mixture in 50 cc PVC bag

Table 3. 8

Mean percent remaining for zidovudine and ranitidine controls and mixtures in 5 % dextrose injection in 50 cc PVC bags stored at 4 degrees Celsius up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	100 ± 0.00	99.30 ± 0.01	98.8 ± 0.8	100.7 ± 0.60
2[b]	100 ± 0.00	100.6 ± 0.01	101.4 ± 1.20	99.9 ± 0.30
3[c]	100 ± 0.00	102.2 ± 0.50	101.4 ± 0.20	98.8 ± 0.60
	100 ± 0.00	100.8 ± 0.50	99.1 ± 2.30	98.8 ± 0.30
4[c]	100 ± 0.00	101.0 ± 0.30	99.9 ± 0.60	94.80 ± 0.60
	100 ± 0.00	98.9 ± 1.00	99.6 ± 1.50	99.4 ± 0.40
5[c]	100 ± 0.00	101.2 ± 0.50	102.8 ± 0.8	97.70 ± 2.20
	100 ± 0.00	99.7 ± 1.00	96.2 ± 0.40	97.80 ± 0.40

a. 2mg/mL zidovudine control in 50 cc PVC bag

b. 0.5mg/mL ranitidine control in 50 cc PVC bag

c. 2mg/mL zidovudine and 0.5mg/mL ranitidine in a mixture in 50 cc PVC bag

Table 3.9

Mean percent remaining for zidovudine and ranitidine controls and mixtures in 0.9 % sodium chloride injection in 50 cc PVC bags, stored at ambient temperature (23 ± 2 degrees Celsius) up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	100 ± 0.00	100.2 ± 0.10	100.8 ± 0.70	101.2 ± 0.80
2[b]	100 ± 0.00	100.7 ± 1.3	100.6 ± 1.00	101.30 ± 0.10
3[c]	100 ± 0.00	100.3 ± 0.6	100.4 ± 0.90	99.4 ± 0.70
	100 ± 0.00	101.0 ± 0.70	101.0 ± 1.10	100.3 ±1.00
4[c]	100 ± 0.00	100.2 ± 0.80	100.7 ± 0.20	99.5 ± 1.30
	100 ± 0.00	100.8 ± 1.6	101.3 ± 0.20	100.9 ± 1.70
5[c]	100 ± 0.00	100.4 ± 0.1	100.5 ± 0.70	99.9 ± 1.0
	100 ± 0.00	100.1 ± 1.80	100.9 ± 0.50	100.0 ± 0.70

a. 2mg/mL zidovudine in control in 50 cc PVC bag

b. 0.5mg/mL ranitidine in control in 50 cc PVC bag

c. 2mg/mL zidovudine and 0.5mg/mL ranitidine in a mixture in 50 cc PVC

Table 3.10

Mean percent remaining for zidovudine and ranitidine controls and mixtures in 5 % dextrose injection in 50 cc PVC bags, stored at ambient temperature (23 ± 2 degrees Celsius) up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	100 ± 0.00	100.4 ± 0.20	99.8 ± 0.40	95.2 ±1.00
2[b]	100 ± 0.00	99.8 ± 0.70	91.5 ± 0.90	91.60 ± 1.30
3[c]	100 ± 0.00	97.4 ± 0.30	93.5 ± 0.60	98.80 ± 0.50
	100 ± 0.00	94.6 ± 0.90	93.80 ± 0.70	91.60 ± 1.60
4[c]	100 ± 0.00	99.10 ± 0.70	94.6 ± 0.80	94.10 ± 0.50
	100 ± 0.00	93.2 ± 1.30	92.10 ± 1.10	93.0 ± 0.70
5[c]	100 ± 0.00	98.5 ± 0.40	93.9 ± 0.30	93.70 ± 1.10
	100 ± 0.00	93.80 ± 1.80	92.5 ± 2.00	91.80 ± 0.90

a. 2mg/mL zidovudine control in 50 cc PVC bag

b. 0.5mg/mL ranitidine control in 50 cc PVC bag

c. 2mg/mL zidovudine and 0.5mg/mL ranitidine in a mixture in 50 cc PVC bag

Table 3. 11

Mean percent remaining for zidovudine and ranitidine controls and mixtures in 0.9 % sodium chloride injection in 50 cc PVC bags, stored at 4 degrees Celsius up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	100 ± 0.00	101.5 ± 0.30	101.6 ± 0.10	99.70 ± 0.80
2[b]	100 ± 0.00	100.0 ± 0.40	96.7 ± 1.10	99.50 ± 0.70
3[c]	100 ± 0.00	100.3 ± 0.70	100.5 ± 0.60	99.30 ± 0.50
	100 ± 0.00	101.6 ± 0.20	96.4 ± 0.70	100.60 ± 0.60
4[c]	100 ± 0.00	99.7 ± 0.60	99.7 ± 0.60	98.60 ± 0.20
	100 ± 0.00	101.3 ± 0.40	99.5 ± 1.00	100.80 ± 0.40
5[c]	100 ± 0.00	100.2 ± 0.80	100.0 ± 0.50	99.60 ± 0.30
	100 ± 0.00	101.3 ± 0.20	100.0 ± 1.40	100.70 ± 0.60

a. 2mg/mL zidovudine control in 50 cc PVC bag

b. 0.5mg/mL ranitidine control in 50 cc PVC bag

c. 2mg/mL zidovudine and 0.5mg/mL ranitidine in a mixture in 50 cc PVC bag

Table 3.12

Mean percent remaining for zidovudine and ranitidine controls and mixtures in 5 % dextrose injection in 50 cc PVC bags stored at 4 degrees Celsius up to 24 hr.

Bag	0 hr	4 hr	8 hr	24 hr
1[a]	100 ± 0.00	100.2 ± 0.10	100.8 ± 0.70	101.2 ± 0.80
2[b]	100 ± 0.00	100.7 ± 1.3	100.6 ± 1.00	101.30 ± 0.10
3[c]	100 ± 0.00	100.3 ± 0.6	100.4 ± 0.90	99.4 ± 0.70
	100 ± 0.00	101.0 ± 0.70	101.0 ± 1.10	100.3 ±1.00
4[c]	100 ± 0.00	100.2 ± 0.80	100.7 ± 0.20	99.5 ± 1.30
	100 ± 0.00	100.8 ± 1.6	101.3 ± 0.20	100.9 ± 1.70
5[c]	100 ± 0.00	100.4 ± 0.1	100.5 ± 0.70	99.9 ± 1.0
	100 ± 0.00	100.1 ± 1.80	100.9 ± 0.50	100.0 ± 0.70

a. 2mg/mL zidovudine in control in 50 cc PVC bag

b. 0.5mg/mL ranitidine in control in 50 cc PVC bag

c. 2mg/mL zidovudine and 0.5mg/mL ranitidine in a mixture in 50 cc PVC bag

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CHAPTER 4

SUMMARY

The objective of this study was to establish the stability of zidovudine when administered with either ranitidineb hydrochloride and or dobutamine hydrochloride as an admixture stored at ambient temperature and 4° C. All three drugs are chemical bases, and the use of a low pH buffer in the mobile phase will increase the retention time on the HPLC system.This explains why dobutamine hydrochloride has the longest retention time because it is the most basic of all the drugs. Zidovudine is intermediate in basicity and that's the reason why it has a retention time between that of ranitidine hydrochloride and dobutamine hydrochloride.The use of a polyamide C_{16} column with amide groups contributed to increased retention due to hydrogen bonding between the sample and the stationary phase, but that was offset by a highly polar mobile phase (84:16 v/v) for zidovudine / dobutamine hydrochloride and (88:12 v/v) for zidovudine / ranitidine hydrochloride, as the polar sample would tend to associate with the predominantly polar mobile phase, and show decreased retention times. All the drugs eluted before 15 min on the amide column.The resolutions were greater than 1.5 and no peaks appeared except for the solvent front peak. An isocratic elution was favoured over a gradient elution in this study because a gradient elution requires cumbersome equipment and software and requires extra time for column equilibration between runs. The 25 mM concentration was an appropriate concentration for a phosphate buffer , as buffers tend to precipitate at high concentrations. Zidovudine injection contained phenol as a preservative and that didn't interfere with the chromatography. Acetonitrile lacks absorbance at the

wavelengths at which the detector was set at 265nm and 280 nm for both experiments, since its UV cutoff is 205 nm. The use of a UV detector would make the detector liable to miss co-eluting peaks, a flow rate of 1mL/min was suitable under these conditions because this was an isocratic run and it would reduce the run times even though the peaks were sharp and symmetrical.

Stability can always be increased by lowering the temperature,but drug formulations or mixtures should not be frozen. Generally drugs are more stable at 4° Celcius than at ambient temperature.This was shown by the areas under the curve remaining more stable at 4° Celcius than at ambient temperature. At ambient temperature there was a significant loss of zidovudine or ranitidine hyrochloride concentration in the 5 % dextrose injection (up to 10 % loss of initial drug concentration for the mixtures (see table 4) than 0.9% sodium chloride injection. The controls in the 5% dextrose injection didn't lose much , which portends to the fact that admixing of the drugs had some effect on the stability of the drugs, but more important is the injection solution itself which is not buffered. The pH values fluctuated between 0 and 24 hr by as much as 0.7 units, which were negligible.The drugs tended to degrade more in the 5 % dextrose solution pH range (4.5-7.0) and less in the 0.9% sodium chloride solution, pH range (5.50-6.50). In the case with dobutamine hydrochloride (pH 2.5-5.50) the higher pH could lead to alkaline hydrolysis. It seems like pH dependent degradation is taking place at high temperatures and is more accentuated in 5 % dextrose.

In conclusion, zidovudine can be admixed for up to 24 hr with ranitdine hydrochloride or dobutamine hydrochloride at ambient temperature and 4° Celsius in 50cc PVC bags.