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Design and evaluation of extemporaneous formulations for treating pulmonary hypertension in children.

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Abstract

Introduction: Children, being the most vulnerable group, whose health status require urgent attention, are constantly in need of extemporaneous formulations for the treatment of several clinical conditions, such as pulmonary hypertension (PH)

Method: Amiloride hydrochloride/ Hydrochlorothiazide (AH) combination, (1mg/ 10mg)/mL, and furosemide, 1mg/mL, were separately formulated into liquid dosage forms using simple syrup BPC, commercially available ascorbic acid syrup, deionized water and deionized water: propylene glycol (ratios 75:25, 65:35 and 50:50) as solvents. The formulations were analyzed for stability over a 7-day period at different storage conditions (27±2°C protected from and exposed to sunlight, and refrigeration at 4±1°C) using physical parameters, pH analysis, UV spectrophotometric assay and microbial count as assessment parameters.

Results: Significant changes were observed for formulations exposed to sunlight (27°±2°C), while refrigerated formulations were the most stable to physical changes, but had increased viscosity. All formulations had reduction in pH values, however, formulations containing deionized water and propylene glycol were the most significant, with formulations protected from light at 27°±2°C providing the least pH changes. Refrigerated formulations retained higher medicament percentages within official limits, while formulations exposed to light (27°±2°C) had the highest loss of potency. Virtually all the formulations prepared with simple syrup BPC aided growth of lactose fermenters. Refrigerated formulations resisted microbial growth most. In terms of stability, the storage conditions can be ranked as 4°±1°C > 27°±2°C protected from light > 27°±2°C exposed to light.

Conclusion: The refrigerated extemporaneous formulations were the most physically stable. Deionized water: propylene glycol (75:25) is the most appropriate solvent for formulating the formulations and should be used only for a maximum of 6 days. It is recommended that extemporaneous formulations containing Amiloride hydrochloride-Hydrochlorothiazide (AH) combination or furosemide are preferably stored at 4°±1°C protected from light, as this offers good resistance to microbial growth.

Keywords: *Extemporaneous preparations, Children, Amiloride hydrochloride-Hydrochlorothiazide combination, frusemide, storage conditions.*

Résumé

Introduction : Les enfants, qui constituent le groupe le plus vulnérable et dont l'état de santé appelle une attention urgente, ont constamment besoin de formulations extemporanées pour le traitement de plusieurs affections cliniques telles que l'hypertension artérielle pulmonaire.

Méthode : Une combinaison d'hydro-chlorure d'amiloride et d'hydrochlorothiazide (AH) (1 mg / 10 mg) / mL et de furosémide, 1 mg / mL, a été formulée séparément dans des formes posologiques liquides en utilisant du sirop simple BPC, du sirop d'acide ascorbique disponible commercialement, et de l'eau désionisée : propylène glycol (rapports 75 : 25 ; 65 : 35 et 50 : 50) en tant que solvants. La stabilité des formulations a été analysée sur une période de 7 jours dans différentes conditions de stockage (27 ± 2 ° C protégés et exposés au soleil, et réfrigération à 4 ± 1 ° C) en utilisant des paramètres physiques, une analyse du pH, un dosage spectrophotométrique UV et une numération microbienne comme paramètres d'évaluation.

Résultats : Des changements significatifs ont été observés pour les formulations exposées à la lumière solaire (27 ° ± 2 ° C), alors que les formulations réfrigérées étaient les plus stables aux changements physiques, mais avaient une viscosité accrue. Toutes les formulations présentaient des valeurs de pH réduites. Cependant, les formulations contenant de l'eau désionisée et du propylène glycol étaient les plus significatives, les formulations protégées de la

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lumière à 27 °C ± 2 °C fournissant le moins de changements de pH. Les formulations réfrigérées conservaient des pourcentages de médicament plus élevés dans les limites officielles, tandis que les formulations exposées à la lumière (27 ° ± 2 ° C) présentaient la perte de puissance la plus élevée. Pratiquement toutes les formulations préparées avec du sirop simple BPC ont contribué à la croissance des fermenteurs de lactose. Les formulations réfrigérées ont le plus résisté à la croissance microbienne. En termes de stabilité, les conditions de stockage peuvent être classées de 4 °± 1°C> 27 °± 2 °C à l'abri de la lumière> 27 °± 2 °C exposées à la lumière.

Conclusion : Les formulations réfrigérées extemporanées étaient les plus stables physiquement. Eau désionisée : propylène glycol (75 : 25) est le solvant le plus approprié pour la formulation des formulations et ne doit être utilisé que pendant 6 jours au maximum. Il est recommandé de conserver les formulations extemporanées contenant une association de chlorhydrate d'amiloride et d'hydrochlorothiazide (AH) ou du furosémide à une température de 4 ± 1 °C, à l'abri de la lumière, comme ceci offre une bonne résistance à la croissance microbienne.

Mots-clés : *Préparations extemporanées, enfants, combinaison d'hydro-chlorure d'amiloride et d'hydrochlorothiazide, furosémide, conditions de stockage.*

Introduction

A significant quality of any extemporaneously prepared formulation is the assurance of the safety and efficacy of the pharmaceutical product in relation to a specified use, which has been reviewed and accepted by an official expert body [1]. In most cases, extemporaneous formulations are intended to satisfy a medicine or dosage form requirement that is not commercially available. A working definition of extemporaneous compounding is the mixing together of the ingredients in a prescription or drug formula, usually performed by a pharmacist to fill an individual order of a drug product that will be used for a relatively short period of time [2].

Children, being the most vulnerable group, whose health status requires urgent attention [3] are constantly in need of extemporaneous formulations for the treatment of several clinical conditions that do not have registered dosage forms for their treatment. This lack of drug formulations suitable for children is a worldwide concern, taken into consideration by some developed countries, as well as by organizations such as the World Health Organization [4]. This is further buttressed by the fact that pharmaceutical companies are sometimes reluctant to study drugs in children because of the complexity, difûculty, and cost of such trials [5]. However, the formulation of licensed drugs as extemporaneous preparations for children use has become common practice in our hospitals and community pharmacies [6, 7]. This development is based on therapeutic advances and adventure that has necessitated the compounding of drugs to suit the clinical needs of such patients. The common dosage form for children is liquid preparations; hence, almost all extemporaneous preparations for children are usually in this dosage form, thus, necessitating the need to ensure the delivery of requisite quality and safe dose consistently to the patient [2].

Pulmonary hypertension is a normal and necessary state for the fetus, however, when cardiopulmonary transition (characterized by a rapid fall in pulmonary vascular resistance and pulmonary artery pressure, and a rise in pulmonary blood flow) fails to occur at birth, then pulmonary hypertension becomes an issue of utmost emergency [8]. Pulmonary hypertension (PH), in children is a rare disease that may lead to morbidity and mortality in this vulnerable group. Unfortunately, the lack of sufficient controlled studies in children makes PH management challenging in paediatrics, with most therapeutic strategies based on extrapolation from adult studies and expert consensus [9]. In Nigeria, the commonest therapeutic approach to the treatment of paediatric PH is the use of first line PH drugs which are formulated extemporaneously on a child-by-child basis.

Diuretics (Amiloride hydrochloride-Hydrochlorothiazide (AH) combination, and furosemide) are the most prescribed of all the first line PH drugs [10, 11] and are readily available as tablets for adult use [12]. Therapeutic advances and adventure has necessitated the extemporaneous compounding of AH and furosemide in the treatment of PH in children. This study is aimed at examining the stability (physical, chemical and microbial) and content uniformity of extemporaneous formulations of Amiloride-hydrochlorothiazide combinations and Frusemide for use in children less than five years of age.

Materials and methods

Materials

Commercially available brands of combined 5mg amiloride hydrochloride and 50 mg hydrochlorothiazide tablets, 40 mg frusemide tablets,

ascorbic acid syrup (100mg/ml) and granulated sugar were procured from a registered pharmacy in Ibadan, Nigeria. De-ionized water, freshly prepared simple syrup (BPC), distilled water and analytical grade propylene glycol were obtained from the research laboratories of the Centre for drug discovery, development and production, University of Ibadan, Ibadan, Nigeria. Nutrient agar, petri dishes and agar plates were procured and made available by the Molecular Laboratory, University of Ibadan, Ibadan, Nigeria. All other reagents used were of analytical grade.

Methods

Preparation of extemporaneous formulations:
Powdered tablets equivalent to 1.0 mg amiloride hydrochloride and 10 mg hydrochlorothiazide per ml of the different solvents [Simple syrup BPC, commercially available ascorbic acid syrup, deionized water (boiled for 15 minutes and cooled prior to usage) and deionized water: propylene glycol (ratios 75:25, 65:35 and 50:50)] were prepared by trituration. The entire procedure was repeated for formulations containing 1mg/ml of frusemide for each solvent. Sufficient quantity of all the formulations were kept in transparent air tight bottles and stored at three different conditions: 27°±2 °C protected from light, 27°±2 °C exposed to light and 4°±1°C over a 7-day period.

Physical examination:
The formulations from the three storage conditions were examined daily on days 0 to 6 for changes in colour, smell, redispersibility and turbidity.

Evaluation of the hydrogen potency of the formulations:
The change in the hydrogen potency (pH) of the extemporaneous formulations over the 7-day period was monitored using a Mettler Toledo pH meter (Mettler Toledo instruments and services, Greinfensee, Switzerland). The pH meter was set to the pH Mode and the temperature was adjusted to the working temperature of the electrode. The electrode was removed from the storage buffer solution, rinsed with de-ionized water and wiped dry. The electrode was then placed in the extemporaneous formulation to be tested, and the pH reading was taken after the display unit stabilized. The operation was conducted in triplicates daily over a 7-day period for all the formulations at the different storage conditions. The electrode was carefully rinsed with de-ionized water, wiped and returned to the storage buffer solution.

Ultra-violent spectroscopic assay of drug content

Assay of drug content of amiloride hydrochloride-hydrochlorothiazide (AM-HCT) formulations: The method developed by Abdelaleem *et al* [13] was adopted to determine the percentage of API in the AM-HCT tablets used for the formulations. Standard working solutions of hydrochlorothiazide and amiloride hydrochloride were prepared in the respective solvents and also in methanol at a concentration of 1 mg/ml. Accurate aliquots equivalent to (40 -200 µg) and (20 - 160 µg) of hydrochlorothiazide and amiloride hydrochloride respectively were transferred from their working solutions into a series of 10 ml volumetric flasks and volumes were completed to the mark with methanol (or the respective solvents) mixed well. Absorbance of the spectra of laboratory-prepared mixtures containing different ratios of amiloride hydrochloride and hydrochlorothiazide hydrochloride were measured at 361 nm corresponding to the contents of amiloride hydrochloride only, and at 277.2 nm corresponding to the total content of amiloride hydrochloride and hydrochlorothiazide in the mixture, using a UV Spectrophotometer (Shimadzu UV spectrophotometer UV-VIS model UV-1280). The concentration of amiloride hydrochloride alone and the total concentration of the two drugs were calculated from their corresponding regression equations. By subtracting amiloride hydrochloride concentration from the total mixture concentration, the actual concentration of hydrochlorothiazide in the mixture was obtained. The assay was carried out for all the extemporaneous formulations containing amiloride hydrochloride-hydrochlorothiazide at different storage conditions on days 0 to 6 (7 days).

Assay of drug content of frusemide formulations:
The method described by Naveed *et al* [14] was adopted to determine the percentage of API in the frusemide tablets used for the formulations. The absorption maximum for frusemide was obtained at 276nm. Standard stock solution containing accurately weighed 5mg of furosemide was transferred to a volumetric flask and sufficient solvent (used in preparing the extemporaneous sample undergoing assay) was added to produce 50ml. This was further diluted to obtain 1mg/ml of frusemide. Several serial dilutions of the standard solution were made and scanned at the wavelength of 276nm to obtain the calibration curve. Volume exactly equal to 1ml of the extemporaneously prepared formulations were also serially diluted and scanned at the wavelength of 276nm and the strength of the frusemide was extrapolated from the

calibration curve obtained. The assay was carried out for all the extemporaneous formulations containing frusemide at different storage conditions on days 0 to 6 (7 days)

Each colony forming unit (CFU) per mL of extemporaneous formulations (and the control formulations) was computed using equation (i): CFU/mL

Table 1: List of abbreviations

Abbreviation	Description of Abbreviation
F/AA	Frusemide tablet dissolved in commercially available ascorbic acid syrup (1mg/mL)
F/DW	Frusemide tablet dissolved in deionized water (1mg/mL)
$F/DW_{75}PG_{25}$	Frusemide tablet dissolved in deionized water: propylene glycol, ratio 75:25 (1mg/mL)
$F/DW_{65}PG_{35}$	Frusemide tablet dissolved in deionized water: propylene glycol, ratio 65:35 (1mg/mL)
$F/DW_{50}PG_{50}$	Frusemide tablet dissolved in deionized water: propylene glycol, ratio 50:50 (1mg/mL)
F/SS	Frusemide tablet dissolved in simple syrup BPC (1mg/mL)
M/AA	Amiloride-hydrochlorthiazide tablets in commercially available ascorbic acid syrup (1.0mg/10mg)/mL
M/DW	Amiloride -hydrochlorthiazide tablets dissolved in deionized water (1.0mg/10mg)/mL
$M/DW_{75}PG_{25}$	Amiloride -hydrochlorthiazide tablets dissolved in deionized water: propylene glycol, ratio 75:25 (1.0mg/10mg)/mL
$M/DW_{65}PG_{35}$	Amiloride -hydrochlorthiazide tablets dissolved in deionized water: propylene glycol, ratio 65:35 (1.0mg/10mg)/mL
$M/DW_{50}PG_{50}$	Amiloride -hydrochlorthiazide tablets dissolved in deionized water: propylene glycol, ratio 50:50 (1.0mg/10mg)/mL
M/SS	Amiloride -hydrochlorthiazide tablets dissolved in simple syrup BPC (1.0mg/10mg)/mL
NIL	No colony forming units observed
TNC	Colony forming units are too numerous to count

Any code with an extension of' _C "(in the microbial analysis table) is serving as the control of that code

Microbial analysis of the extemporaneous formulations

The extemporaneous formulations were observed for growth of microbial colonies. Total bacterial counts, coliform counts as well as presumptive *Salmonella* counts for the formulations were ascertained using the viable count technique over a 7 day period. Sampling was carried out on days 0, 1, 5 and 7. Exactly 0.1 mL of each formulation was serially diluted in 9.9 mL of 0.85 % normal saline. Exactly 0.1 mL of appropriate dilutions was aseptically inoculated into different plates containing nutrient agar, MacConkey lactose fermenting (LF) agar and MacConkey non-lactose fermenting (NLF) agar, and spread evenly on the agar surface with the aid of a pre-sterilised glass spreader. The dilution was increased as the days progressed. Formulations containing only the solvent without the active pharmaceutical ingredients were also prepared to serve as controal and subjected to the same conditions described for the extemporaneous formulations. Plates were then incubated at 37°C for 24 hours after which plates were observed and the colony forming units (per mL) were counted and computed.

$$\frac{\text{Number of colonies counted per plate x dilution factor}}{\text{Volume of inoculum (=0.1)}}$$

Eqn (i)

Results and Discussion

Physical examination

Formulation failures in extemporaneously prepared oral suspensions usually result from physical incompatibilities, drug-excipient interaction issues and drug degradation. An insoluble drug suspended in a suitable vehicle may be less susceptible to drug degradation, but may settle out of the suspension over time, leading to sedimentation and caking. In this state, there will be a higher concentration of drug at the bottom of the bottle than at the top. If taken as it is, this will result in the patient being under dosed at the beginning and overdosed towards the end of a treatment course [15] (Tucker *et al*, 2010). In order to ensure uniformity of dose, these formulations need to be shaken properly before use and patients need to be adequately counseled.

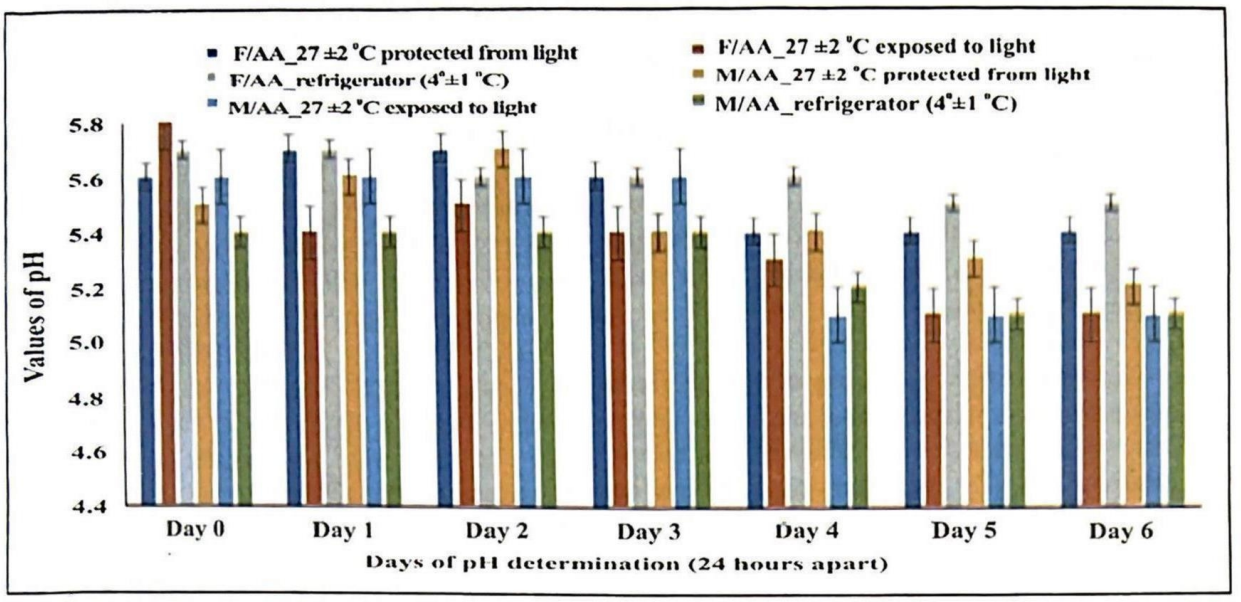

Fig. 1: Plot showing the effect of storage condition on pH of extemporaneous formulations prepared with ascorbic acid syrup.

Significant changes were generally observed for formulations stored at 27°±2 °C exposed to sunlight, while formulations that were refrigerated were the most stable to changes in physical appearance, but had an observed increase in viscosity. Takeomo *et al* [16] documented that the rate of chemical degradation usually increases with temperature, a factor which is the basis for accelerated stability trials of pharmaceutical formulations. Thus, exposure to sunlight could have been responsible for the pronounced changes noticed in the formulations stored at 27°±2 °C exposed to sunlight. The relatively insignificant changes observed for the formulations refrigerated (4°±1 °C) is justifiable because, at low temperatures the rate of chemical reaction slows and physical appearance tends to remain unchanged [17]. However, formulations prepared using different ratios of deionized water: propylene glycols were observed to be relatively stable at all storage conditions, with the following physical stability ranking: $DW_{75}PG_{25}$ < $DW_{65}PG_{35}$ < $DW_{50}PG_{50}$ In 2015, Nalawade *et al* [18] observed that the presence of propylene glycol in a medium enhances the stability, thus providing an explanation for the relative stability as the content of the propylene glycol was increased. Generally, the formulations containing ascorbic acid as the solvent were noticed to become the most difficult to re-disperse with time. This is due to the highly viscous nature of the solvent. Patel *et al* [5] reported that loss of re-dispersibility of liquid formulations may create an impression of loss of efficacy.

Evaluation of potency of hydrogen (pH)

Hydrolysis, oxidation and reduction are the most common reactions usually associated with liquid preparations [19]. Usually the reaction rate or type is influenced by pH. The presence of excipients may reduce chemical stability of extemporaneous formulations prepared from tablets by changing the pH to a value at which more rapid degradation occurs. Thus, optimal pH is therefore required to maintain stability [19]. All the formulations had a reduction in pH values; an indication that the formulations had increased acidity with time. However, formulations prepared using different ratios of deionized water and propylene glycol had the most significant reduction in pH values. The storage condition that provided the least changes in pH was 27°±2 °C protected from light, with the ranking for frusemide formulations as $F/DW_{50}PG_{50}$ > $F/DW_{75}PG_{25}$ > F/DW = F/SS > $F/DW_{65}PG_{35}$ > F/AA, while those containing amiloride-hydrochlorthiazide ranked $M/DW_{65}PG_{35}$ > $M/DW_{50}PG_{50}$ > M/DW > M/SS > M/AA, thus showing that the most acidic solvent was ascorbic acid as shown in Fig. 1. It was observed that the formulation containing frusemide stored at 4°±1°C (refrigerated) had the highest pH value, while frusemide formulations stored at 27°±2°C (exposed to and protected from sunlight) and amiloride-hydrochlorthiazide formulations stored in the refrigerator had the least pH values.

The significance of pH changes in extemporaneous formulations cannot be overemphasized. An extemporaneous preparation may have its safety profile compromised as a result of significant changes in pH, which could lead to therapeutic failures and subsequently hospital admission [5]. An alkaline product could become acidic and vice-versa, and since most extemporaneous formulations rarely contain buffers, pH changes must remain insignificant at the storage conditions of the formulations.

Ultra-violent spectroscopic assay of drug content

Extemporaneous preparations are often given arbitrary shelf-lives or shelf-lives based on published information [20]. It is important to ensure that an extemporaneous formulation packaged in a specific container will remain within its physical, chemical and microbiological specifications during storage for a specified time [21]. A major goal of extemporaneous formulations is to ensure that the active pharmaceutical ingredient (API) remains within the stipulated quantity throughout the duration of use [22]. Practically, the shelf life can be assumed to be the time taken for the concentration of the drug to be reduced to 95% of its value when originally prepared [21]. On the other hand, a reduction of content down to 90% of theoretical value (with possible 95% confidence bounds) is generally regarded as the maximum reduction acceptable [23]. Thus, the limit of ≥90% of the initial concentration is also acceptable for extemporaneous formulations [24].

Assay of the formulation were carried out spectrophotometrically and some results are represented in Fig. 2. Formulations stored in the refrigerator (4°±1°C) retained the highest percentage of the APIs (F/AA > F/SS > $M/DW_{75}PG_{25}$ > M/SS > F/DW > $M/DW_{65}PG_{35}$ > $M/DW_{50}PG_{50}$ > $F/DW_{65}PG_{35}$ > M/DW > M/AA > $F/DW_{50}PG_{50}$ > $F/DW_{75}PG_{25}$) as shown in Fig. 2, while formulations stored at 27°±2 °C exposed to light had the highest loss of potency (F/AA > F/DW > M/DW > $F/DW_{75}PG_{25}$ > $M/DW_{75}PG_{25}$ > F/SS > $M/DW_{50}PG_{50}$ > $F/DW_{50}PG_{50}$ > M/SS > $F/DW_{65}PG_{35}$ > M/AA > $M/DW_{65}PG_{35}$). For the frusemide formulations stored in the refrigerator (4°±1°C), the formulation containing deionized water: propylene glycol (75:25) as solvent had the highest loss of potency (From 99.45±0.23% to 81.13 ±1.16%). This further establishes the report that propylene glycol has the ability of restraining changes in the medium where it is prevalent [18]. Relatively, formulations containing deionized water as solvent were able to retain the percentage of the APIs above the limit of ≥ 90% (Fig.2) as proposed by Glass and Haywood (2006). This reflects that the absence of specific ions (in de-ionized water), helped to maintain the shelf life of the formulations at all the storage conditions evaluated. The use of commercially available ascorbic acid syrup (containing antioxidants) and simple syrup BPC (containing a high glucose content) did not offer any

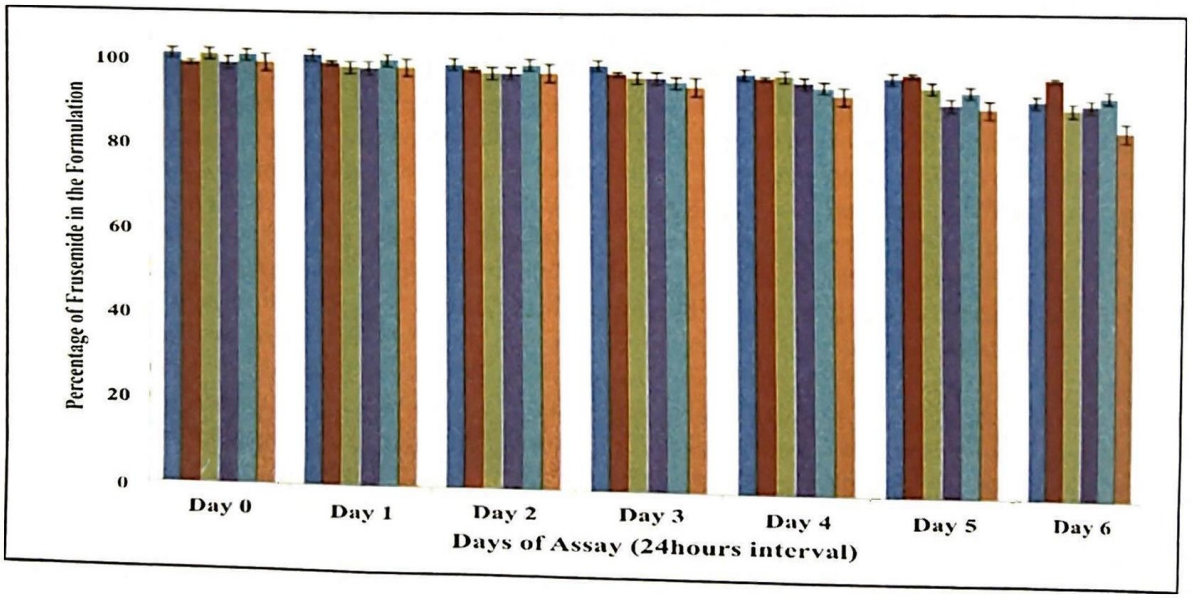

Fig. 2: Representative Plot showing percentage of frusemide in extemporaneous formulations stored at 4°±1 °C (refrigerated).

Table 2: Microbial evaluation of formulations stored at 4°±1 °C (refrigerated): Day 6 count

Sample	CFU/mL(Nutrient Agar)				CFU/mL(MacConkey Agar_LF)				CFU/mL(MacConkey Agar_NLF)			
Dilution Factor	10^{-1}	10^{-2}	10^{-4}	10^{-6}	10^{-1}	10^{-2}	10^{-4}	10^{-6}	10^{-1}	10^{-2}	10^{-4}	10^{-6}
F/AA	1600	5000	1	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
F/AA_C	500	2000	1	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
F/DW	4400	11000	8	2	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
F/DW_C	1400	3000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$F/DW_{75}PG_{25}$	1900	4000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$F/DW_{75}PG_{25}$_C	4400	9000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$F/DW_{65}PG_{35}$	5300	11000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$F/DW_{65}PG_{35}$_C	900	7000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$F/DW_{50}PG_{50}$	2600	7000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$F/DW_{50}PG_{50}$_C	800	9000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
F/SS	1600	11000	500000	30000000	TNC	TNC	TNC	120000000	700	5000	400000	40000000
F/SS_C	100	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
M/AA	600	1000	NIL	NIL	NIL	NIL	NIL	NIL	3	NIL	NIL	NIL
M/AA_C	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
M/DW	2300	11000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
M/DW_C	1200	10000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$M/DW_{75}PG_{25}$	1900	8000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$M/DW_{75}PG_{25}$_C	1400	13000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$M/DW_{65}PG_{35}$	1600	8000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$M/DW_{65}PG_{35}$_C	1100	9000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$M/DW_{50}PG_{50}$	1200	10000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
$M/DW_{50}PG_{50}$_C	1100	9000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
M/SS	2400	14000	100000	10000000	TNC	TNC	TNC	10000000	NIL	NIL	NIL	NIL
M/SS_C	1100	9000	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

advantage as the APIs still degraded in these solvents.

Microbial analysis of extemporaneous formulations

The assessment of the microbiological content of non-sterile formulations is important in view of the fact that microbes can reduce the therapeutic efficiency of the formulation or introduce infections to the consumer of such products. It becomes even more pertinent for extemporaneous formulations that contain oral tablets that are not originally expected to be in liquid medium prior to administration. Thus, at every stage of formulation, products should be prevented from microbial contamination. The extemporaneous formulations were observed for microbial growth over a 7-day period using nutrient agar, MacConkey (Lactose fermenting,LF) agar and MacConkey (Non-Lactose fermenting, NLF) agar as the bacteria growth substrates. MacConkey agar is a selective and differential medium designed to isolate and differentiate enterics based on their ability to ferment lactose. The crystal violet and bile salts present inhibits the growth of gram-positive organisms which allows for the selection and isolation of gram-negative bacteria. Enteric bacteria that have the ability to ferment lactose can thus be detected. Lactose Fermenters (LF) such as *Escherichia coli, Citrobacter* and *Klebsiella* may be isolated using MacConkey (LF) agar, while lactose non-fermenters (LNF) such as Salmonella may be isolated using the MacConkey (NLF) agar.

The formulations that were refrigerated resisted the growth of microbes more than the other formulations. In terms of formulation stability, the storage conditions can be ranked as 4°± 1°C > 27°± 2°C (protected from light) > 27°± 2°C (exposed to light). The control formulations had no growth on the first day of microbial analysis, however, growth was observed towards the last two days for the control formulations, with special reference to formulations stored at 27°± 2°C exposed to ligh as shown in Table 3t. Growth of lactose fermenting microbes was observed for relatively all the formulations prepared with simple syrup BPC, while formulations prepared with commercially available ascorbic acid had the highest observed resistance to microbial growth.

Conclusion and Recommendation

Physical stability of the formulations is best at storage condition of 4°±1 °C (refrigerated), while deionized water/propylene glycol (75:25) is the most appropriate solvent for the formulations and should be used only for a maximum of 6 days. Quantitative estimation of the active pharmaceutical ingredients in the extemporaneous

formulations indicate that the presence of propylene glycol has the ability of restraining changes in the medium, and subsequently retarding loss of potency within 7 days. Refrigeration of extemporaneous formulations containing frusemide or amiloride-hydrochlorothiazide combinations at 4°±1 °C protected from light offer good resistance to microbial growth.

It is recommended that more research into different solvents for use in extemporaneous formulations containing different medicaments be carried out, to enable community and hospital pharmacists have a data base of compatible solvents and medicaments. This is especially useful for targeted patient groups, such as children, whose dosage forms are often in this category.

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