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Article

Stability of furosemide and aminophylline in parenteral solutions

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Parenteral solutions (PS) are one of the most commonly used drug delivery vehicles. Interactions among the drug, components in the drug's formulation, and the PS can result in the formation of inactive complexes that limit efficacy or increase side effects. The aim of this work was to evaluate possible interactions between the drugs and PS, assess drug stability and to identify degradation products after 20 h at room temperature. Furosemide (FSM) and Aminophylline (APN) were added to PS containing either 20% mannitol or 0.9% NaCl at pH 6.5-7.5 and 10-11. Their behavior was studied individually and as an admixture, after 1 h oxidation with H_2O_2, using a spectrophotometer and HPLC. Individually, FSM and APN added to 20% mannitol and 0.9% NaCl solutions had the highest stability at pH 10-11. When FSM and APN were combined, the behavior of FSM was similar to the behavior observed for the drug individually in the same solutions. With the drugs combined in 20% mannitol pH 10-11, HPLC showed that both drugs were stable after a 20 h period yielding two distinct peaks; in oxidized samples, the elution profile showed four peaks with retention times unrelated to the untreated samples.

Uniterms: Parenteral solutions. Spectrophotometer. High Performance Liquid Chromatography. Furosemide. Aminophylline.

Soluções parenterais de grande volume são frequentemente utilizadas no ambiente hospitalar para a veiculação de fármacos. No entanto, possíveis incompatibilidades entre as estruturas dos fármacos, em diferentes veículos de administração, podem gerar possíveis associações antagônicas ou sinérgicas, resultando em alterações das propriedades físico-químicas, consequentemente, dos efeitos farmacológicos e das respostas clínicas esperadas. Este artigo avaliou a estabilidade e a possível formação de produtos de degradação entre os fármacos furosemida e aminofilina quando estes foram veiculados em soluções parenterais, após o preparo e após o período de 20 h. Furosemida e aminofilina foram adicionadas às soluções de 20% manitol e 0,9% NaCl nos valores de pH 6,5-7,5 e 10-11. A estabilidade dos fármacos foi avaliada individualmente, combinada e após degradação com peróxido de hidrogênio através de espectrofotometria de UV e HPLC. Furosemida e aminofilina individualmente avaliadas mostraram alta estabilidade em ambas as soluções estudadas nos valores de pH 10-11. Quando os fármacos foram combinados o comportamento da furosemida foi similar ao observado na ausência de aminofilina. Os fármacos combinados em 20% manitol pH10-11 por HPLC foram estáveis após o período de 20 h. Após degradação o perfil de cromatograma encontrado foi diferente do observado na ausência de degradação mostrando que o método é indicativo de estabilidade.

Uniterms: Soluções parenterais. Espectrofotometria. Cromatografia líquida de alta eficiência. Furosemida. Aminofilina.

INTRODUCTION

Parenteral solutions (PS) are one of the most commonly used drug delivery vehicles. The investigation of drug stability in parenteral solutions and their formulations is important in the administration and efficacy of any treatment. Interactions among drugs, components in the drug's formulation, and parenteral solutions can result in the formation of inactive complexes that limit efficacy or increase side effects.

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The addition of drugs to parenteral solutions, either individually or combined, alters the physical and chemical properties of these solutions. The key parameters are changes in pH, in ion concentrations and the addition of solvents.

Furosemide (FSM) is a potent diuretic widely used in the treatment of edema associated with heart and renal disorders (Katzung *et al*., 1998). Furosemide primarily inhibits sodium and chloride reabsorption in the thick ascending limb of the loop of Henle (Florence, Attwood, 2003) promoting increased elimination of potassium, magnesium, calcium and, to a lesser extent, bicarbonates (Florence, Attwood, 2003; Goodman *et al.,* 2005).

Furosemide is an acidic (pKa = 3.9), white to slightly yellowish solid commercially available diluted in basic solutions, using NaOH to increase its water solubility (Thomas, Altman, 1987). It is unstable at acidic pH (Martindale, 1993) undergoing hydrolysis and photodegradation (Hitoshi *et al.*, 1995) into furfuryl alcohol and 4-chloro-5-sulfamoylanthranilic acid; the furfuryl alcohol decomposes further to levulinic acid and other decomposition products, as elucidated by Kovar *et al*. (1974).

The stability of furosemide in water and aqueous solutions, composed of NaOH, HCl, $(NH_4)_2HPO_4$, phosphate buffers, sorbitol and other components, were examined at temperatures from 24 °C to 85 °C over 7 to 182 days by Ghanekar *et al*. (1978). Their results showed that furosemide stability was related to pH and temperature of the solutions proving unstable in acidic media and very stable in basic media. The addition of alcohol to 50% sorbitol solutions at pH 8.5 stabilized furosemide for up to 182 days at 24 °C.

Kirit *et al*. (1980) studied the effects of pH, chlorobutanol, cysteine HCl, EDTA, propylene glycol, sodium metabisulfite and sodium sulfite in aqueous solutions (25 °C), on furosemide stability. These authors reiterated that the critical parameter in furosemide stability was pH, with the highest stability at basic pH throughout the shelf life of aqueous solutions of furosemide.

As an admixture, furosemide was shown to be compatible with ceftazidime in parenteral nutrient solution for 24 h (25 °C) (Servais, Tulkens, 2001) and stable in a solution of 25% human albumin for 48 h at 25 °C and 14 d at 4 °C (Elwell *et al.*, 2002).

Theophylline, a naturally occurring methylxanthine derivative, competitively inhibits phosphodiesterase, resulting in increasing amounts of cyclic AMP (cAMP) which promotes the release of endogenous epinephrine. Increased levels of cAMP may also inhibit the release of histamine and of slow reacting substance of anaphylaxis (SRS-A) (Dawson *et al.,* 1986).

Aminophylline, a 2:1 complex of theophylline and ethylenediamine, has two distinct actions: smooth muscle relaxation (bronchodilation) and suppression of the response of the airways to stimuli (non-bronchodilator effects). It is generally administered by continuous intravenous infusion when used as a potent bronchodilator in the treatment of lung diseases (Korolkovas, 1988).

Aminophylline was shown to be stable in alkaline solutions (optimal pH 8.6-9.0) but dissociates into theophylline and ethylenediammonium ions at pH 2.4-3.0. In the presence of CO_2, aminophylline dissociates into theophylline (Dawson *et al.*, 1986) and upon exposure to light and oxygen decomposes into 1,3-dimethylallantoin, *N*,*N*'-dimethyloxamide and ammonia (Ishiguro *et al*., 1991).

Aminophylline was shown to be stable for 24 h in parenteral nutrient solutions (PN) (25 °C) at concentrations ≤1.5 mg/mL (16) and PN in DIMIX bags (Ciszewska-Jedraski *et al.*, 1995), and for 91 days in Ora Sweet:Ora Plus oral suspension with 3 mg/mL (4 °C and 25 °C) and 21 mg/mL (25 °C) (Chong *et al.*, 2000).

As an admixture (constant infusion method), aminophylline (1-2 mg/mL) with ceftazidime was incompatible in both 0.9% NaCl and 5% dextrose (25 °C) (Pleasants *et al.*, 1992), compatible with cimetidine HCl in 5% dextrose for 48 hours (25 °C) (Baptista, Miltrano, 1988) and compatible with fluconazole in 0.9% NaCl and 5% dextrose for 3 h (25 °C) (Jonhson *et al*., 1993).

An admixture of furosemide and aminophylline in parenteral solutions increases the diuretic effect of furosemide in cardiac patients, compared with the use of furosemide alone. A binding study showed that small doses of aminophylline administered to patients, after utilization of furosemide, can increase the diuretic effect of furosemide in children with cardiac pathology (Mclaughin *et al*., 2000).

Furosemide is listed as an essential drug and aminophylline as a supplementary drug by the World Health Organization (WHO, 2009). Although newer drugs are available, furosemide and aminophylline are routinely prescribed in both developing (Mendis *et al*., 2007) and developed countries (Kaufman *et al*., 2002) due to their low cost and efficacy. The clinical importance of furosemide is evidenced by the large number of analytical procedures available to detect the presence of this drug in pharmaceutical and physiological sample.

Since the inherent chemical stability of a drug molecule largely determines the stability of the final pharmaceutical product, regulatory authorities require novel chemical entities (NCE) to undergo extensive chemical stability evaluation (stress testing) in accordance with the internationally accepted guidelines of the International Conference

on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Therefore, the evaluation of drug stability in parenteral solutions, individually or associated, is necessary to guarantee therapeutic effectiveness with minimum adverse effects, ensuring the patient's safety and adequate treatment. In this sense, the evaluation of furosemide and aminophylline as an admixture is urged due to its current application in routine clinical practice as an intravenous injection.

The purpose of this work was to evaluate possible interactions between the drugs and parenteral components, and to assess drug stability over 20 h at room temperature. The 20 h duration was selected because this period was determined to be the maximum storage time from mixing to administration in Brazilian clinical practice. Furosemide and aminophylline were added to parenteral solutions of 20% mannitol and 0.9% NaCl, and their behavior studied individually and as an admixture using a spectrophotometer and HPLC as per the methods proposed by Ruiz-Angel (Ruiz-Angel *et al.*, 2006).

MATERIAL AND METHODS

Glassware and equipment

All glassware was immersed in a 50% ethanol solution of 1 M NaOH for 24 h, drained and transferred to a solution of 1 M nitric acid for 6 h, rinsed for 24 h in purified water (Milli-Q) and autoclaved for 30 min at 121°C.

The pH of all solutions was measured with a pHmeter (Accumet AR20, Fisher Scientific, USA) and absorbance was measured using a spectrophotometer (Beckman DU-640, USA) and 1 cm quartz cuvettes. For all assays, samples were assessed at 25 °C.

Solutions preparation

A 4 mg/mL stock solution of furosemide (FSM; Medley S/A Indústria Farmacêutica, São Paulo State, Brazil) was made by dissolving into a solution of 32.5 mM NaOH in water for injection (WFI) with a final pH of 9.5±0.5. A 24 mg/mL stock solution of aminophylline (APN; Medley S/A Indústria Farmacêutica, São Paulo State, Brazil) was made in WFI to yield a stock solution with a final pH of 9.5±0.5. Stock solutions were prepared with all solvents at room temperature (25 °C) immediately prior to use.

An aliquot of the drug stock solutions was added individually or combined to either 250 mL of WFI or 250 mL of commercially available parenteral solutions (PS) of either 20% mannitol or 0.9% NaCl (Aster Pharmaceutics Labs, Ltd., São Paulo State, Brazil) to a final concentration of 1.6 mg/mL for FSM and 0.96 mg/mL for APN; solutions in WFI were used to generate calibration curves. The solutions were prepared and stored in glass beakers covered with plastic wrap in triplicate, as follows: (i) 80% parenteral solution with 16% FSM stock solution and 4% WFI (solvent for APN), (ii) 80% parenteral solution with 4% APN stock solution and 16% WFI+NaOH (pH 9-10, solvent for FSM), (iii) 80% parenteral solution with 16% FSM and 4% APN stock solutions. Samples were assayed at pH 6.5-7.5 (pH obtained after successive dilutions with parenteral solutions without adjustment) or pH 10-11 using a solution of 32.5 mM NaOH to minimize further dilution of the drug, mannitol or salt concentrations.

The mixing ratios and storage conditions emulated routine conditions in the hospital setting. Assaying at pH 6.5-7.5 was done to emulate final drug/parenteral solutions for patient use. In aqueous solution, FSM and APN are most stable at pH 10-11; adjustment of the pH to 10-11 was done to compare the solutions of these drugs at the pH with optimal stability.

Immediately after mixing and after 20 h storage at 25 °C, protected from light, the pH and absorbance of these solutions were measured at 25 °C. The absorbance was measured at 228 and 275 nm for FSM and APN, respectively, and also at both wavelengths for solutions containing the drugs combined (Santos *et al.*, 2007).

HPLC

Reversed-phase HPLC was performed on a Shimadzu LC 10 with photodiode detector (PDA SPD-M10A; software LC solution; Shimadzu, Japan) using a 250 x 4.6 mm (i.d.) column pre-packed with C_{18} (Shim-pack VP–ODS, Shimadzu, Japan) with 0.6 mL/min flow at 25 °C and a sample injection volume of 20 µL; the mobile phase consisted of 30 % acetonitrile and 70% phosphate buffer (pH 7.0) and eluted samples were detected at 228 nm and 275 nm.

Oxidation

Immediately after mixing, triplicate 3 mL aliquots of each drug/PS solution at pH 10-11 were exposed to a strong oxidant, drug/PS solution:10% H_2O_2 (2:1, v/v), to promote drug degradation and to evaluate if the degradation products could interfere with the sample analysis by HPLC. After 1 h oxidation with H_2O_2, triplicate 20 µL samples in solution of 20% mannitol were analyzed by HPLC and compared to untreated samples. No residual

H_2O_2 was detected after 1 h contact with the drug/PS solutions (30).

RESULTS AND DISCUSSION

Absorbance Scans

Standard solutions of FSM and APN in WFI, and in parenteral solutions (PS) of 0.9% NaCl and 20% mannitol (pH 7.0±0.5), were used to generate the calibration curves. The absorbance calibration curves for FSM (λ = 228 nm) using commercial (Hypofarma Instituto de Hypodermia e Farmácia S/A., Brazil**)** and standard drug **(**Medley S/A Indústria Farmacêutica, São Paulo, Brazil**)** ranged from 2 - 10 μg/mL. The calibration curve for APN (λ = 275 nm; purity ≥99%, Medley S/A Indústria Farmacêutica, São Paulo, Brazil) ranged from 2.4 -19.2 μg/mL. The equations derived from the commercial drug curves were used for calculating drug concentrations; the calibration results using the commercial drugs were selected to relate this assay to compounds used in the clinical setting.

Furosemide Stability

Furosemide in 20% mannitol (pH 10-11) showed higher absorbance values after 20 h (initial concentration 1.6 mg/mL versus 2.47 mg/mL after 20 h) with this difference attributed to changes in furosemide molecular structure in the presence of mannitol. Upon immediate mixing, FSM in 0.9% NaCl (pH 10-11) showed a decrease in concentration to 1.47 mg/mL compared to initial concentration (1.60 mg/mL). After 20 h, the concentrations of FSM were closer to initial values (1.52 mg/mL) showing that FSM was stable in 0.9% NaCl parenteral solutions for up to 20 h.

FSM stability in 20% mannitol solution at pH 10-11, as measured by absorbance after a period of 20 h, showed a standard deviation higher than 5% (p>5%) and therefore the obtained data was not conclusive.

FSM in 20% mannitol (pH 6.5-7.5) showed initial concentrations of 2.46 mg/mL, an increase compared to the amount added to the solution (1.60 mg/mL). After 20 h concentrations remained high at 2.44 mg/mL. Initial absorbance readings of FSM in 0.9% NaCl (pH 6.5-7.5), showed concentrations of 1.80 mg/mL dropping after 20 h to 1.38 mg/mL.

Aminophylline stability

Initial concentrations of APN in 20% mannitol (pH 10-11) were measured at 1.01 mg/mL, values that were closer to the calculated 0.96 mg/mL at initial mixing. After 20 h, APN concentrations in these solutions decreased to 0.80 mg/mL. In solutions of 0.9% NaCl (pH 10-11), initial APN concentrations were 0.96 mg/mL whereas after 20 h the APN concentrations dropped to 0.93 mg/mL. These results showed that APN was stable in both parenteral solutions at pH 10-11.

In solutions of 20% mannitol (pH 6.5-7.5), APN showed initial concentrations of 0.9 mg/mL compared with 0.85 mg/mL after 20 h. APN in 0.9% NaCl (pH 6.5-7.5) showed initial concentrations of 0.99 mg/mL whereas after 20 h the concentration was 0.92 mg/mL. These results show that APN is stable in both solutions at neutral pH.

Stability of furosemide and aminophylline admixtures

At a pH of 10-11, APN in the presence of FSM, in both 0.9% NaCl and 20% mannitol solutions, showed an increase in initial concentrations and also after 20 h. This increase could be attributed to the contribution of a minor absorbance of FSM at the same wavelength used to detect APN (λ = 275 nm), hampering an accurate determination of APN stability in the presence of FSM using a spectrophotometer. Therefore, FSM stability was examined in samples containing FSM and APN combined in either the mannitol or NaCl solutions (both at pH 10-11) and analyzed after initial preparation and after 20 h storage by HPLC. Although peak retention times for APN were consistent either alone or combined with FSM, as an admixture, APN stability could not be verified by concentration using HPLC. This was because FSM contributed to the absorbance values in the wavelength used to detect APN, consistently yielding higher concentrations for APN than the calculated amount. The results were compared to the absorbance readings of the standards and for the individual drugs immediately after preparation and after 20 h.

Immediately after mixing of FSM and APN in 20% mannitol solutions (pH 10-11) observed concentrations were 99 μg/mL for APN and 170 μg/mL for FSM, higher than the calculated addition of 96 μg/mL (APN) and 160 μg/mL (FSM), but within limits of experimental error (<10%). As an admixture of FSM and APN in 20% mannitol solutions (pH 10-11), FSM initial stability and after 20 h was similar to FSM alone in 20% mannitol. Combined with APN, similar results were observed for FSM in 0.9% NaCl solutions (pH 10-11) to those seen with the drug alone in this solution.

Ghanekar *et al*. (1978) examined the stability of FSM in different solutions at various concentrations of sugar and observed that increasing absorbance values

correlated with changes in pH as the sugar concentrations increased. These results could be related to the increase in absorbance for FSM in mannitol in the pH 10-11 and pH 6.5-7.5 ranges observed in this study. Christensen (1983) studied the stability of FSM in oral liquid drug products and also reported a loss in stability in acidic medium and sucrose solutions.

HPLC elution profiles

In this study, the HPLC methodology was validated in accordance with the guidelines contained in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – ICH, 2005 (33). The parameters evaluated were specificity, linearity, range, precision, and accuracy of the methodology, and determination of stress testing using an oxidant.

Validation of the HPLC analyses were proven by determining if FSM and APN can be detected consistently by comparing the elution profiles of the samples with the standard reference solutions of FSM and APN in water for injection (WFI). The retention times for sample solutions and the standards were compared and evaluated (Figures 1, 2, and 3).

Precision was evaluated by testing seven repetitive samplings with 100% of the standard drug concentrations (FSM 160 µg/mL and APN 96 µg/mL; Table 1); linearity was determined by testing drug concentrations ranging from 75 to 150% of standard concentration. Variance, standard deviation and coefficient of variance were also evaluated.

Samples of drugs, alone or combined, in 20% mannitol (pH 10-11) were exposed to a strong oxidant (H_2O_2) and the elution profiles then compared to untreated samples using HPLC (Figures 1-4). For oxidized APN, the elution profiles showed two peaks, but only one corresponded to APN (4.5 min, peak 2), exhibiting the same retention time as untreated APN solutions. The minor peak was not identified. Oxidized FSM solutions showed changes in retention time (9.4 min) compared to untreated FSM solutions (8.4 min). This indicated that after 20 h storage in 20% mannitol, FSM was stable, but significantly modified upon exposure to an oxidant.

With both APN and FSM added to 20% mannitol (pH 10-11), two peaks were obtained, the first peak corresponding to APN (retention time 4.5 min) and the second peak corresponding to FSM (retention time 8.5 min). In the oxidized samples, the elution profile showed four peaks with retention times unrelated to the untreated samples (Figure 4).

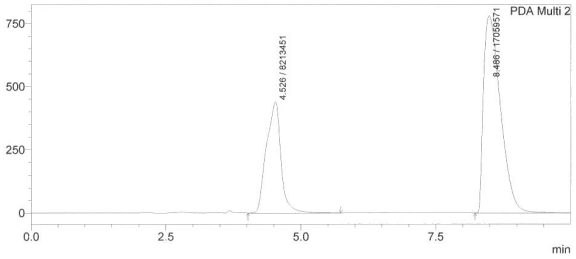

FIGURE 1 - HPLC chromatogram of furosemide (8.5 min) and aminophylline (4.5 min) combined in 20% mannitol (pH 10-11) upon preparation at 25 °C.

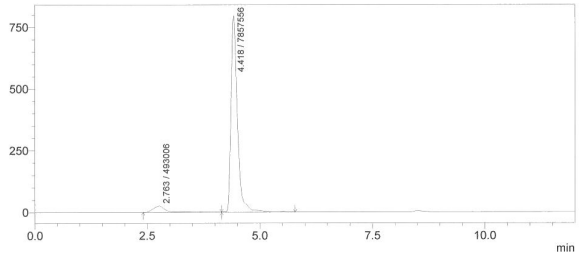

FIGURE 2 - HPLC chromatogram of aminophylline in 20% mannitol solution (pH 10-11) upon preparation at 25 °C.

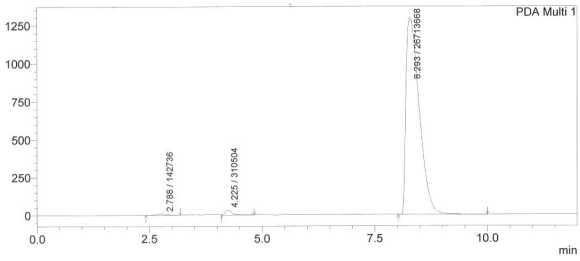

FIGURE 3 - HPLC chromatogram of furosemide in 20% mannitol solution (pH 10-11) upon preparation at 25 °C.

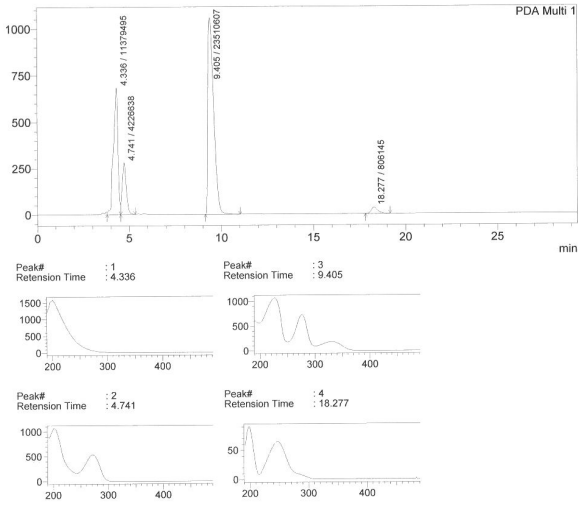

FIGURE 4 - HPLC chromatogram and spectra of eluted samples from FSM and APN combined in 20% mannitol (pH 10-11) after oxidation with H_2O_2.

TABLE I - Precision, standard deviation, variance and %CV of HPLC retention times (minutes) for solutions of furosemide (λ = 228 nm) and aminophylline (λ = 275 nm) in 20% mannitol solutions after 20 h at 25°C, individually and combined

Retention times of FSM and APN admixture.

	Replicate No.	Retention Times	RSD Reference Std Dev	Maximum, Minimum, Mean, Median	Std Dev	Variance	%CV
FSM 228 nm 20% Man pH 10-11	1st	8.486	0.040	8.486	0.0375	0014	0.4449
	2nd	8.442		8.372			
	3rd	8.438		8.417			
	4th	8.405		8.405			
	5th	8.402					
	6th	8.377					
	7th	8.372					
APN 275 nm 20% Man pH 10-11	1st	4.526	0.0047	4.539	0.0043	0.00002	0.0957
	2nd	4.529		4.526			
	3rd	4.537		4.532			
	4th	4.529		4.530			
	5th	4.530					
	6th	4.531					
	7th	4.539					

Retention times of FSM and APN individually in solution.

	Replicate No.	Retention Times	RSD	Maximum, Minimum, Mean, Median	Std Dev	Variance	%CV
FSM 228 nm 20% Man pH 10-11	1st	8.293	0.015	8.303	0.0130	0.0002	0.1570
	2nd	8.274		8.272			
	3rd	8.272		8.286			
	4th	8.303		8.284			
APN 275 nm 20% Man pH 10-11	1st	4.414	0.004	4.417	0.0031	0.00001	0.0698
	2nd	4.417		4.409			
	3rd	4.416		4.414			
	4th	4.409		4.415			

* RSD-Reference standard deviation refers to deviation obtained in analytical data and in this case was the same as Std Deviation, and therefore a single data value was considered.

Individually, peak retention times and concentrations (peak height) for FSM and APN by HPLC were constant after 20 h showing that both drugs are compatible in 20% mannitol (pH 10-11) parenteral solutions. Combined, HPLC showed neither significant changes in the elution profiles nor the presence of other peaks, suggesting that both drugs are compatible as an admixture in 20% mannitol (pH 10-11).

Baptista *et al*. (1988), studied the stability of an admixture of APN and cimetidine HCl in 0.5% glucose parenteral solutions (D5W) by HPLC. Their samples were analyzed after 1, 6, 24 and 48 h with no significant pH changes observed in either the test or control solutions. The study showed that cimetidine HCl (1200 mg) and APN (500 mg) when admixed in 1 L of D5W are both chemically stable and physically compatible for 48 h at room temperature.

Paul *et al.*(1983), studied the stability of APN in three parenteral solutions using HPLC and confirmed it was stable in alkaline media with an optimal pH 8.6-9.0. Aminophylline concentrations lower than 40 mg/mL appear to be stable over a wide pH range (Baptista *et al*., 1988). Admixtures of aminophylline and amino acid solutions added directly to large volume parenteral nutritive solutions in concentrations not exceeding 1.5 mg/mL are stable for 24 h (Ciszewska-Jedraski *et al*., 1995).

CONCLUSIONS

Individually, FSM and APN added to 20% mannitol and 0.9% NaCl solutions had the highest stability at pH 10-11. When FSM and APN were combined in the same parenteral solutions, the behavior of FSM was similar to the behavior observed for the drug individually in the same solutions.

With the solutions at neutral pH, the stability of FSM could not be verified because the concentrations of FSM were higher than the calculated initial concentration. As an admixture with FSM, the stability of APN in parenteral solutions of 20% mannitol and 0.9% NaCl could not be verified because the absorbance of FSM at 275 nm interfered with the detection of APN. Although absorbance measurements are useful for detecting molecular decomposition (blue shift) or the formation of soluble aggregates (red shift or peak broadening), this study clearly reveals that the use of a spectrophotometer was inadequate for FSM at neutral pH and for FSM and APN combined.

Future studies will be conducted assessing APN stability in 20% mannitol and 0.9% NaCl solutions, using revised HPLC methods to further utilize the only method enabling detection of APN in the presence of FSM.

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REFERENCES

BAPTISTA, R. J.; MILTRANO, F. P**.** Stability and compatibility of cimetidine hydrochloride and aminophylline in dextrose 5% in water injection. *Drug Intell. Clin. Pharm.*, v.22, p.592-593, 1988.

CISZEWSKA-JEDRASKI, M.; KNYT, A.; PERTKIEWICZ, M. Aminophylline stability in total parenteral nutrition admixtures. *Acta Pol. Pharm.*, v.52, p.487-490, 1995.

CHRISTENSEN, J. M.; LEE, R.; KEITH, A. P. Stability of three oral liquid drug products repackaged in unit dose containers. *Am. J. Hosp. Pharm.*, v.40, p.612-615, 1983.

CHONG, E.; DUMONT, R. J.; HAMILTON, D. P.; KOKE, M. P.; ENSOM, M. H. H. Stability of aminophylline in extemporaneously-prepared oral suspension. *J. Inform. Pharmacother.*, v.2, p.100-106, 2000.

ELWELL, R. J.; SPENCER, A. S.; BARNES, J. F.; WYNN, C. E. Stability of furosemide in human albumin solution. *Ann. Pharmacother.*, v.36, p.423-426, 2002.

FLORENCE, A. T.; ATTWOOD, D. *Princípios físico-químicos em Farmácia*. São Paulo: Edusp, 2003. 736 p.

GHANEKAR, A. G.; GUPTA, V. D.; GIBBS, C. W. J. Stability of furosemide in aqueous systems. *J. Pharm. Sci.*, v.67, p.808-811, 1978.

GOODMAN, L.; GILMAN, A. *The pharmacological basis of therapeutics*. New York: Mc Graw-Hill, 2001. p.45-65.

HITOSHI, S.; NAOMI, Y.; EMIL, T. L.; LESLIE, Z. B. Apparent intramolecular acyl migration and hydrolysis of furosemide glucuronide in aqueous solutions. *Biol. Pharm. Bull.,* v.18, p.134-139, 1995.

INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE. Validation of Analytical Procedures: Text and Methodology Q2(R1). 2005. Available at: http://www.bioforum.org.il/Uploads/Editor/karen/q2_r1_step4.pdf. Accecced on: 15 jun. 2007.

ISHIGURO, Y.; SAWADA, M.; TANAKA, Y.; KAWABE, K. Photo-stability of aqueous aminophylline solutions under oxygen. *J. Liq. Chromatogr. Relat. Technol.,* v.100, p.1048-1053, 1991.

JHAJ, R.; GOEL, N. K.; GAUTAM, C. S.; HOTA, D.; SANGEETA, B.; SOOD, A.; SACHDEV, A. Prescribing patterns and cost of antihypertensive drugs in an internal medicine clinic. *Indian Heart J.,* v.53, p.323-327, 2001.

JOHNSON, C. F.; JACOBSON, P. A.; PILLEN, H. A.; WOYCIK, C. L. Stability and compatibility of fluconazole and aminophylline in intravenous admixtures. *Am. J. Hosp. Pharm.*, v.50, p.703-706, 1993.

KAUFMAN, D. W.; KELLY, J. P.; ROSENBERG, L.; ANDERSON, T. E.; MITCHELL, A. A. Recent patterns of medication use in the ambulatory adult population of the United States*:* the slone survey. *JAMA, J. Am. Med. Assoc.*, v.287, p.337-344, 2002.

KATZUNG, B. G. Interações importantes entre fármacos. In: __, (Ed.). *Farmacologia*. Rio de Janeiro: Guanabara Koogan, 1998. p.242-259.

KIRIT, A.; SHAH, V. G.; KENNETH, R. S. Effect of pH, chlorobutanol, cysteine hydrochloride, ethylenediaminetetraacetic acid, propylene glycol, sodium metabisulfite, and sodium sulfite on furosemide stability in aqueous solutions. *J. Pharm. Sci.,* v.69, p.594-596, 1980.

KOROLKOVAS, A. B.; JOSEPH, H. *Química farmacêutica.* Rio de Janeiro: Guanabara Koogan, 1988. p.111-119.

KOVAR, K. A.; WOJTOVICZ, G. P.; AUTERHOFF, H. Die hydrolytische Spaltung einiger Sulfonamid-Diuretika. *Arch. Pharm.*, v.307, p.657-662, 1974.

MARTINDALE*:* The Extra Pharmacopoeia. London: The Pharmaceutical Press, 1993. 2363 p.

MCLAUGHLIN, G. E.; LAND, M. P.; ROSSIQUE-GONZALEZ, M. Effect of aminophylline on urine flow in children with tacrolimus induced renal insufficiency. *Transplant. Proc.*, v.32, p.817-820, 2000.

MENDIS, S.; FUKINO, K.; CAMERON, A.; LAING, R.; FILIPE, A.; KHATIB, O.; LEOWSKI, J.; EWEN, M. The availability and affordability of selected essential medicines for chronic diseases in six low- and middle-income countries. *Bull. W. H. O.,* v.85, p.279-289, 2007.

PAYING THE PRICE: A 19-state survey of the high cost of prescription drugs. Available at: <http://www.yuricareport.com/Medicare/WisOurFutureOnMedicare.pdf>. Accessed on: 28 fev. 2011.

PAUL, W.; NIEMIEC, T. W. J. R.; VANDERVEEN, M. W.; HOHENWARTER, R. H. Stability of aminophylline injection in three parenteral nutrient solutions. *Am. J. Hosp. Pharm.,* v.40, p.428-432, 1983.

PLEASANTS, R. A.; LEIGHT, M.; VAUGHAN, D. M.; WILLIAMS, J. L. F. Compatibility of ceftazidime and aminophylline admixtures for different methods of intravenous infusion. *Ann. Pharmacother.,* v.26, p.1221-1226, 1992.

PONTO, L. L.; SCHOENWALD, R. D. Pharmacokinetics and pharmacodynamics of furosemide in protein-calorie malnutrition. *Clin. Pharmacokinet.*, v.18, p.381-408, 1990.

RUIZ-ANGEL, M. J.; BERTHOD, A.; CARDA-BROCH, S.; ÁLVAREZ-COQUE, M. C. G. Analytical techniques for furosemide determination. *Sep. Purif. Rev.*, v.35, p.39-58, 2006.

SANTOS, C. A.; MAZZOLA, P. G.; SILVA, P. H. S.; CHOLEWA, O.; PENNA, T. C. V. Preliminary study on the potential utility of GFP as a biosensor for drugs stability in parenteral solutions. *Biotechnol. Prog.,* v.23, p.979-984, 2007.

SERVAIS, H.; TULKENS, P. M. Stability and compatibility of ceftazidime administered by continuous infusion to intensive care units. *Antimicrob. Agents Chemother.*, v.45, p.2643-2647, 2001.

THOMAS, R. E.; ALTMAN, P. M. *The pharmacy practice foundation of the University of Sydney. Medical Remedial Enlistment Program (Medrep manual)*. Sydney: Medrep, 1987. p.3.90-3.98, p.7.24-7.28.

WETTERMAKR, B. *A study on the range of drugs and the quality of prescribing in the different European Union member states using the DU90% method*. Available at: http://www.euromedstat.cnr.it/pdf/copenhagen/07_wettermark.pdf. Accessed on: 26 abr. 2006.

WORLD HEALTH ORGANIZATION. *Model list of essential medicines*. EML 15: 15th list. Available at http://www.who.int/medicines/publications/08_ENGLISH_indexFINAL_EML15.pdf. Accessed on: 21 mar. 2010.

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