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In vivo release study of a developed buoyant dosage form using Furosemide as a model drug

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ABSTRACT

In-vivo evaluation of the developed buoyant dosage form in dogs resulted in a significant increase in Furosemide systemic availability (1.3 times) compared to conventional tablet. Based on the in vitro and in-vivo investigation findings, buoyant tablets might be considered as a feasible approach in delivering Furosemide; however, such feasibility needs to be verified among large population of human subjects.

Key words: *buoyant, in vivo, in vitro.*

INTRODUCTION

Furosemide is an anthranilic acid derivative is one of the most potent and frequently used loop diuretic. The oral bioavailability from an immediate release tablet (IR) is poor (60%) and highly variable due to the presence of a biological window comprised of the upper gastrointestinal tract. The purpose of the present study is to develop and optimize a modified release floating tablet for Furosemide with an increased gastric residence time, and to assess the *in vivo* release and drug availability compared to an immediate release dosage form.

METHODOLOGY

For convenience, dogs were selected as experimental animals for the comparative in-vivo investigation of the selected developed dosage forms and conventional immediate release **(Dalton and Meyer, 2002)** where a total of 8 young local breed dogs (average weight of 10 ± 2 kg) were classified into two groups and housed separately under controlled environment (25 ± 2 °C and 55% relative humidity).

Determination of Furosemide in dog plasma:

Modified form of the method reported earlier by **Lin etal., (1979)** was used where 0.2ml of the obtained plasma was introduced into centrifuge tube containing 0.2ml each of methanol, perchloric acid (3.5%), sulphamethoxazole (5µg/ml) as internal standard. The mixture was then shaken manually and centrifuged at 3000 rpm for 20 minutes and aliquots of 20µl of the supernatant was injected into HPLC comprise of Knauer variable length U.V. detector and Knauer 64 mobile phase delivery system set at 1.5 ml/min. Column used was Eurospher-100 C_{18} 5µ, 250 × 4.6mm at room temperature. The eluent was monitored at 280nm with detector sensitivity set at 0.01 AUFS. The mobile phase was methanol: 0.01M sodium acetate, pH 5.0 (35:65 v/v) prepared by mixing 350 ml of methanol with 650 ml of purified water followed by addition of 0.6ml of glacial acetic acid. The final pH of the solution was adjusted to 5.0 with addition of 0.3ml of 4*M* NaOH solution. The prepared mobile phase was filtered and sonicated for 1hr prior use. Under such condition no peak corresponds to Furosemide was observed in the control blank sample, moreover, retention time for Furosemide and internal standard peaks were 10.47 and 5.71 min, respectively (**Fig. 1a-c**).

Calibration curve of Furosemide in dog plasma:

Calibration curve was constructed from drug-free plasma samples spiked with increasing concentration of the drug and constant concentration of the internal standard (0.2ml of 5µg/ml solution). Plasma samples were then processed as mention under above and injected into HPLC. Quantification was made using the peak area ratio (PAR) of Furosemide and the internal standard to the concentration of Furosemide This relation was found to be linear in Furosemide concentration range of 2.5-50µg/ml with linear equation of Y = 0.0451X - 0.1524 and correlation coefficient (r) of 0.996 (**Fig.2**). Validation of the method concerned with precision and reproducibility was verified using the criteria of coefficient of variation (CV%) at intra and inter day replication of analysis (**Table 1**).

Dosage forms administration:

Based on the results of the in vitro investigation three developed formulae (F66, F67, F68 which have shown good buoyancy results has been selected to investigate the *in vivo* release tendency of Furosemide from tablets of the invention and to elaborate the role of dosage form buoyancy on the attained drug availability in systemic circulation compared to that of the conventional immediate release tablets.

A latin square cross over expermintal design with 4 runs and 2 weeks washing period was applied under fasting conditions. Each dog was given one of the investigated formulations (including IR) with 50 ml of water under fasting conditions preceded by overnight fasting. The given dose was adjusted to 40 mg Furosemide, and dosage forms tested were a brand of Furosemide available in the local market (40 mg/ tablet) as a conventional immediate release tablet and the developed floating tablets of formulations (each contains 40mg Furosemide/ tablet). Half an hour prior dosage form administration all dogs was given 1 gm ascorbic acid in aqua solution to lower the gastric pH level. With an exception to water, fasting conditions were maintained up to 4 hour post-dosing. At designed time intervals, 3 ml venous blood samples (cephalic, femur, and jugular vein) was withdrawn and collected in vacutainers containing sodium citrate as anticoagulant. Blood samples were then centrifuged (3000 rpm for 20 minutes) and collected plasma samples thus obtained were kept in a refrigerator at -20° C till analysis (**Menon, 1994)**.

Analysis of samples was performed and results concerned with attained level of Furosemide in plasma from different formulation tested were graphed in Figures 3a and 3b. Descriptive parameters of the in-vivo profiles of Furosemide were shown by different formulations and accompanying statistical analysis of variance were summarized in **Tables 2 & 3**, respectively.

Calibration curve of Furosemide for *in vivo* application:

Calibration curve of reference standard Furosemide was generated in dog plasma (**Fig. 2**).

Relation of peak area ratio (PAR) of Furosemide and the internal standard to the concentration of Furosemide was found to be linear in Furosemide concentration range of 2.5-50µg/ml, with linear equation of Y = 0.0451X - 0.1524 and correlation coefficient (r) of 0.996 as described previously. Table 1 show values of CV% for inter and intraday variation that reflects the precision and reproducibility of the analytical method adopted as discussed before. General chromatogram of blank plasma, plasma spiked with internal standard and plasma spiked with internal standard and Furosemide are shown in figures 1a-c, respectively.

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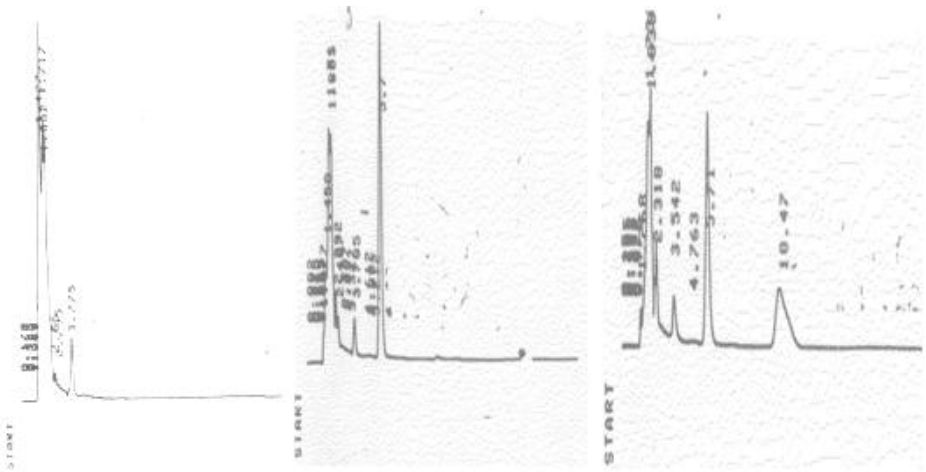
Comparative Furosemide *in-vivo* release profiles:

From figures 3a and 3b, it is obvious that significant difference in Furosemide plasma levels do exist between the formulations investigated ($p < 0.05$, **Tables 2 & 3**). However, attained drug plasma levels from the immediate release (IR) tablets and F-68 were shown to be not significantly different ($p > 0.05$, **Table 3**).



(a) (b) (c)

Fig. 1: Typical chromatogram of (a) dog plasma blank, (b) blank plasma spiked with 5µg/ml sulphamethoxazole as internal standard, and (c) blank plasma spiked with 5µg/ml internal standard and 5µg/ml Furosemide

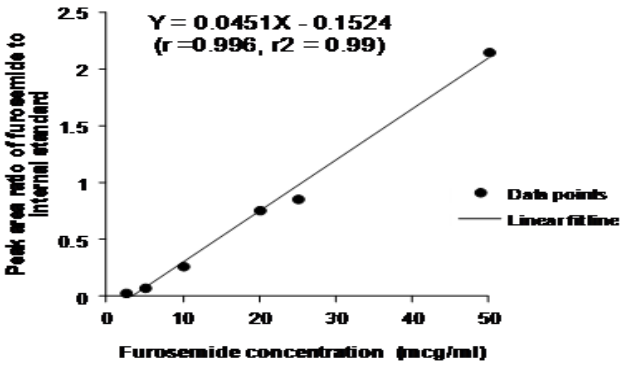


Fig. 2: Calibration curve of Furosemide in fresh dog plasma. Each data point is the average of 3 determinations. Values of SD are not shown

During the first time intervals, IR tablets showed the highest Furosemide plasma levels compared to other formulations (**Fig. 3a**) especially at 1-2hrs post-dose ingestion. This might be attributed to the initial fast release of the drug from IR tablets when the amount of the drug available at absorption site starts to increase. However, 2hrs and forth going, drug plasma levels attained from F66 were apparently the highest with less fluctuation among others till the end of the investigational period. It has been reported that the sustained input of the Furosemide significantly improve diuretic and natriuretic efficiencies during the first 5 hours and thereby increased the total effects measured over 24 hours and improved the pharmacodynamic actions due to the sustained absorption in the stomach and jejunum, which delayed the body's counter activity to the drug effect **(Klausner, et al., 2003)**.

Accordingly, presence of Furosemide at absorption site in large amount might be considered as non-advantageous especially during the first 5 hrs of dose administration where presence of drug in large amount tends to saturate the carrier system so quickly with the remaining being move out of the jejinum in increasing rate based on the gastrointestinal transit time of solution. As a result of this, the fraction of the drug which is supposed to be passively absorbed is significantly reduced since the drug is characterized by absorption window and it is well documented that such absorption window is the main reason behind the low bioavailability of orally administered Furosemide **(Ebihara et al., 1983; Gohary and Gamal, 1991)**.

RESULTS AND DISCURSION

Table No. 1: Values of coefficient of variation (CV%) for inter and Intra-Day Quantitative Determination of Furosemide in plasma

Furosemide concentration (µg/ml)	CV%
Inter-day (n=5)[a]	
2.5	8.3 %
5	6.4 %
20	5.5 %
Intra-day (n=3)[b]	
2.5	9.6 %
25	4.8 %
50	4.7 %

[a] Each value is the average of 5 determinations.
[b] Each value is the average of 3 determinations.

This might answer the question why the absolute enhancement of Furosemide solubility (being sparingly soluble) might not be a promising tool to enhance the oral availability of the drug.

Compared to IR tablets, F66 deliver the drug in a sustain manner, thus providing the drug continuously to its absorption sites in a controlled manner extending the absorption phase of the drug and owing to this drug level in plasma is apparently higher for the respective time interval between the two drug product. This is confirmed by values of C_{max} and T_{max} of Furosemide from IR and F66 tablets shown in **Table 2**.

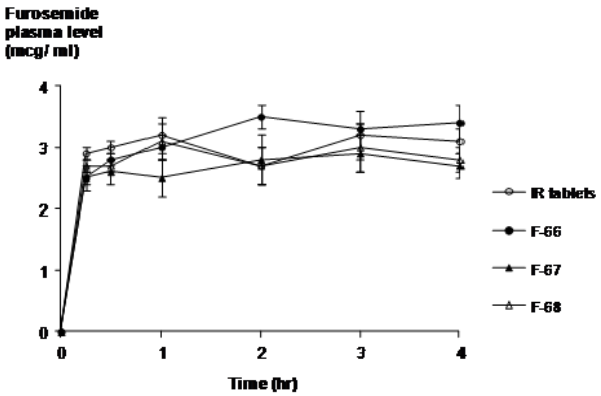


Fig. 3a: Furosemide plasma level attained in dogs from formulations IR, F66, F67 and F68 during the first 4 hrs post administration. Each data point is the average of 3 determinations ± SD

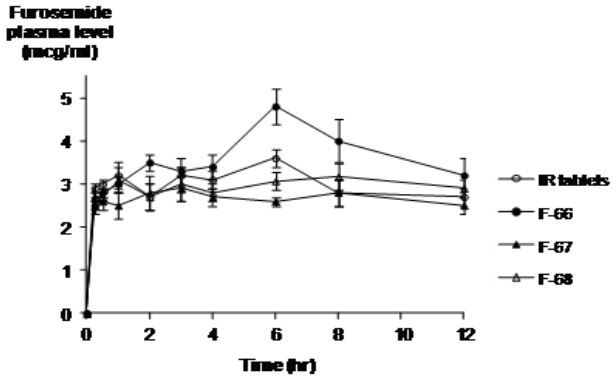


Fig. 3b: Furosemide plasma level attained in dogs from formulations IR, F66, F67 and F68 for 12 hrs post administration. Each data point is the average of 3 determinations ± SD

Table No. 2: Descriptive parameters for in-vivo Furosemide release from immediate release tablets, F66, F67 and F68[a]

Parameters	I.R. Tablets	F66	F67	F68
T_{max} (hr)	1 ± 0.2	2 ± 0.5	3 ± 0.5	3 ±1
C_{max} (mcg/ml)	3.2 ± 0.7	3.5 ± 0.3	2.9 ± 0.4	3 ± 0.5
AUC_{0-12} (mcg.hr/ml)	24.8 ± 3.3	30.42± 4.2	19.2 ± 4.7	23.4±3.1

[a]Data are presented as mean values ± SD.

Table No. 3: Statistical analysis on invivo Furosemide release from from different formulations

Code	Mean	Variance	N
One-way ANOVA Test[a]			
IR*	2.72	0.98844	10
F66	3.05	1.56056	10
F67	2.39	0.72544	10
F68	2.614	0.87487	10
Paired t-Test[b]			
IR	2.72	0.98844	10
F68	2.614	0.87487	10

*Immediate release Furosemide tablets F = 0.72695, p = 0.54261. At the 0.05 level, the means are significantly different. [b]t = 1.28184, p = 0.23193. At the 0.05 level, the two means are NOT significantly different.

In spite of the fact that tablets of F67 exhibited the slowest in-vitro drug release compared to the other formulations, it fails to reveal superiority over IR with respect to the attained in-vivo Furosemide plasma levels (**Fig. 3a & 3b**). It seems there does exist a limit beyond which no enhancement in drug absorption might occur upon further slowing of the drug release from these floating tablets.

Statistical analysis showed no significant difference in the attained drug plasma level between IR and F68 (**Table 3**). However, it is evident from Table 2 that F66 enhances the systemic availability of Furosemide by 1.3 times that of IR as indicated by values of area under plasma-time curve (AUC) for each.

Table No. 4: Appendix

Formula code	HPMC content/ tablet (mg)	Mg. Stearate % ontent	Furosemide content/ tablet (mg)	$NaHCO_3$ content/ tablet (mg)	Hardness (kg/cm^2)	Remarks[a]
F66	40	2%	40	12.00	6.8	Floats immediately
F67	87.5	2%	40	19.50	6.5	Floats immediately
F68	80	2%	40	19.50	6.4	Floats immediately

CONCLUSION

The outcomes of this study highlight the ability to improve the systemic availability of Furosemide by continuous and prolonged input of the drug to the stomach and the upper part of the intestine using the new GRDF which based on buoyancy.

Localization of the developed tablets was not verified and it might be necessary to run some trials to ensure this localization especially when food effect is encountered. Moreover, in-vivo investigations are to be adopted in large population or groups of animal and to be extended to human subjects under both fasting and non-fasting conditions before making the decision with regard to suitability of such tablets for the oral delivery of Furosemide.

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Conflict of interest: The authors have declared that no conflict of interest exists.

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