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Original Article

EPLERENONE FLOATING MICROSPHERES: RADIOGRAPHIC AND PHARMACOKINETIC STUDIES IN RABBITS

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ABSTRACT

Objective: The objective of the present investigation was to evaluate gastro-retentive performance and pharmacokinetic parameters of Eplerenone-optimized floating microspheres compared with formulated floating tablets.

Methods: Microsphere contains antihypertensive drug Eplerenone as a core material encapsulated with the polymeric membrane for sustained drug release were prepared by solvent diffusion-evaporation technique. The prepared microspheres were evaluated for qualitative and quantitative parameters. The optimized formulation showed favorable *in vitro* floating and drug release profile. The gamma scintigraphy of the formulation was carried out in rabbit in order to determine the floating ability of the final formulation with barium sulfate. Prolonged gastric residence time of over 12 h was achieved in all the animals. Eplerenone-loaded optimized formulation was orally administered to rabbit and blood samples were used to determine pharmacokinetic parameter by using WinNonlin software 3.0 version.

Results: Eplerenone floating microsphere, which are compared with pharmacokinetic parameters of the Floating tablet showed improved parameters of Cmax; similarly, time to reach peak plasma concentration (t-max) for Eplerenone Floating microspheres was 4 times increased against Floating tablet formulation. The area under the curve (AUC) for formulated floating tablet was found to be 9.69 µg/ml, whereas for floating microspheres it was 16.28 µg/ml, for formulated floated tablet absorption rate constant Ka was 1.61 h, elimination rate constant was 0.112 h and elimination half-life 6.2 h. The comparison of these data undoubtedly shows that the Cmax was not much valid, but AUC was increased to about 1.68 times in case of floating microspheres, absorption rate constant was found to be decrease 3.22 times when related to the floating microspheres, whereas Ke was found to be decrease 2.11 times when equated to floating microspheres, elimination half-life was increased by almost about two times.

Conclusion: Eplerenone floating microsphere, which are compared with pharmacokinetic parameters of the floating tablets showed enhanced parameters of the formulated due to floating nature of the present designed formulation.

Keywords: Floating microspheres, Gamma scintigraphy, Eplerenone, Pharmacokinetic study, Statistical analysis

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INTRODUCTION

Among the different routes of drug administration, the oral route has achieved the most attention due to the ease of administration and flexibility in dosage form design [1]. Unfortunately, in most cases, the important variability of the gastrointestinal tract physiology and its transit time leads to unpredictable bioavailability and non-reproducible therapeutic effects [2]. One requisite for the successful performance of oral controlled-release drug delivery systems is that the drug should have good absorption throughout the gastrointestinal tract (GIT) [3]. Several difficulties are faced in designing controlled release systems for better absorption and enhanced bioavailability. One of such difficulties is the inability to confine the dosage form in the desired area of the gastrointestinal tract. Drug absorption from the GIT is a complex procedure and subject to many variables [4].

Gastroretentive systems can remain in the gastric region for several hours and, hence, significantly prolong the gastric residence time of drugs. Prolongation of gastric residence time (GRT) of rate-controlled oral drug delivery has shown increased predictability and bioavailability of the dosage form, especially for molecules with a narrow absorption window. Moreover, the total GI transit time is prolonged, thus, the number of dosage regimen can be reduced and solubility can be improved for drugs that are less soluble in a high pH environment [5].

Microspheres have played a key role in the progress of controlled release and gastroretentive systems, as they can encapsulate miscellaneous types of drugs and small molecules, nucleic acids, and proteins [6]. They are biocompatible, can deliver superior bioavailability, and are able to release over longer periods [7]. In addition, microspheres have been technologically advanced by numerous techniques comprising combinations of phase separations or precipitations, emulsion or solvent evaporation, and spraying methods [8]. Floating microspheres are one of the most promising buoyant gastroretentive drug delivery systems. These are free-flowing spherical empty particles without a core, with size varying from 1 to 1000 µm [9]. The GI transit-controlled preparations are intended to float on gastric juice with a specific density of less than one, and due to this property, a delayed transit through the stomach occurs. The slowly released drug at a preferred rate results in enhanced gastric retention with abridged fluctuations in plasma drug concentration [10].

In vitro release studies using conventional and modified dissolution methods can provide insight into the performance of drug-delivery systems, and radionuclides incorporated into the dosage form provide information on the *in vivo* behavior of dosage forms. Gamma scintigraphy is a well-established radionuclide imaging technique [11]. This technique is valuable for evaluating various dosage forms. It is non-invasive and provides reliable information on the transit time of dosage forms in different regions of the GIT and various other body organs. Gamma scintigraphy can analyze the time taken for the disintegration of the drug product and the site where disintegration occurs [12]. The effect of different conditions such as the presence of food, diseased state, and dosage size also can be explored. The process is significantly different from traditional techniques such as diagnostic X-ray methods, where external radiation is passed through the body to form an image. Contrary to this approach, the gamma scintigraphic technique relies on the detection of radiation emitted from radionuclides tagged with dosage forms that are administered intravenously or orally. Release of the tagged tracer is monitored rigorously *in vitro.* Moreover, this technique should be performed in a protected environment [14].

The current study was aimed to formulate Eplerenone floating microsphere and evaluate their gastroretentive performance *in vivo* using gamma scintigraphy.

MATERIALS AND METHODS

Materials

Eplerenone was procured as a gift sample from RA ChemPharma., Hyderabad. Dichloromethane, ethanol, polyvinyl pyrrolidine, and barium sulphate were purchased from SD find Chemicals Mumbai India. Subject animals were acquired from Vab bioscience, Hyderabad.

Preparation of eplerenone floating microspheres

The Eplerenone floating microspheres were prepared by taking drug concentration, dichloromethane: methanol proportion, and stirring time as independent variables to derive the outcomes for particle size, entrapment efficiency, and *in vitro* release as dependant variables. The floating microspheres were prepared by dissolving ethyl cellulose and Eplerenone in optimized proportions of dichloromethane: methanol and adding the resultant solution into 0.75% polyvinyl alcohol [15]. The solution was stirred at definite 500 rpm for prescribed varying stirring time, and the emulsion was filtered with whatman filter paper and washed several times in distilled water; the resulting microspheres were dried and stored in a desiccator. The prepared microspheres were evaluated for yield, entrapment efficiency, buoyancy percentage, particle size, zeta potential, morphology, thermal behavior, physicochemical characteristics (X-ray diffraction), compatibility, *in vitro* release and stability [16].

Preparation and uptake of radio-opaque marker

In the existing study, floating microspheres formulation composition is intended by design expert software with ethyl cellulose of 343.944 mg and drug was substituted with barium sulphate were liquefied in 1 ml DCM and 2.9 ml of methanol; the consequential solution was added dropwise into the water containing 0.75% PVA. The solution was agitated at definite 500 rpm prescribed for 1.45 h. Barium sulphate should be further added in such a way that it should not make microsphere to sink. After the evaporation of the solvent, the emulsion was filtered with Whatman filter paper and washed numerous times in distilled water; the resulted microbeads were dried and stored in desiccator [17].

In vivo gastroretentive study using gamma scintigraphy

To determine the *in vivo* gastroretentive study after oral administration of formulation, imaging analysis is one of the most reliable and accurate tools. Gamma scintigraphy study is one such non-invasive imaging analysis technique [18]. It was performed after approval of protocol by the Institutional Animal Ethics Committee (1687/PO/Re/S/2021/CPCSEA). The *in vivo* radiographic studies were conducted in healthy male Albino rabbits (Vab bioscience, Hyd.) of 2 to 3 kg weight brought and kept to acclimatize for normal day and night cycles for 10 d. During this period they were given with standard diet, rabbits were divided into two groups (group 1 and group 2). Three rabbits were selected for each group to give Barium sulphate-loaded microspheres suspension and formulated floatingtabletin order to examine the floating behavior [18]. Rabbit were fasted for 12 h prior to the study and the dose equivalent of 70 mg of microspheres were given to the rabbit in the form of suspension followed by giving sufficient amount of drinking water. The location of the formulation was identified by gamma scintigraphy by restricting the movement of the animal; they were not allowed to take food until the entire experiment was completed [19].

Pharmacokinetic study

Drug availability study was conducted for prepared Eplerenone floating microspheres compared to commercially available tablets after oral administration to three male albino rabbits having body weight of 2 to 3 kg. These rabbits participated in crossover design and were fasted overnight prior to dosing and given optimized formulation following by water. Blood samples (2 ml) were obtained from the marginal ear vein of the rabbit, which were withdrawn periodically along with the blank (before dosing). The treatment groups were interchanged to develop crossover design [20]. In this design, every rabbit received two doses of formulated floating tablet and floating microspheres. This method was selected to minimize the inter-subject variability. The obtained blood samples were taken in heparinized tube and are subjected to centrifugation; the resulted plasma fraction was transferred by micropipette into the Eppendorf tubes to be frozen immediately at-20 °C until further analysis. WinNonlin version 3.0 software (P Corporation, USA) was used for the measurement of the concentration of drug in plasma overtime [21]. Analysis was done by using non-compartmental method, data were desired from dose administered and statistical moments of the plasma concentration-time profile All the plasma drug concentration were computed based on trapezoid area calculation. The subsequent pharmacokinetic parameters were assessed from the plasma concentration data, and their corresponding peak areas, as shown in table 1 and fig. 4. The subsequent parameters were assessed for floating tablet formulation and floating microspheres. Maximum plasma concentration (Cmax), area under the plasma concentration-time curve from the time of dosing extrapolated to infinity (AUC0-α), absorption rate constant (h), elimination rate constant (h), required lag time to elicit pharmacological action, time to reach peak plasma concentration (h) and elimination half-life (h) were estimated from the plasma concentration data [22].

High-performance liquid chromatography (HPLC) analysis in blood samples

Selection of wavelength by UV-spectrophotometry

Appropriate wavelength for HPLC studies was determined by UV spectrophotometer. Serial dilution of Eplerenone to obtained 10 µg/ml concentration was prepared, where it shows the highest solubility as per the literature review; the above concentration was scanned in the range of 400-200 nm. The maximum spectra were obtained at 240 nm [23].

Chromatographic condition

Analysis was carried out using HPLC equipped with Shimadzu LC (Liquid chromatography)-20 AD Toyoko Japan. Solvent delivery pump equipped with a 20 µl loop and Rheodyne sample injector, Hypersil RP-c18 column (250 mm x 4.6 mm) purosphur star uncapped (5 µm particle size) Narmstadt Germany. Thermoscientific analytical column was used. The detector used was SPD-20A Toyoko Japan dual-wavelength UV-visible detector and elute was measured at 240 nm. The sensitivity was set at 0.0001 AUFS. A 20 µl Hamilton syringe was used for sample injection at isocratic flow rate was kept at 1 ml/min, the data were recorded using LC software solution version 2.1 LC.

Preparation of mobile phase

The mobile phase was prepared by mixing acetonitrile and water in the ratio of 50:50 v/v and later it was sonicated for 10 min for the removal of bubbles. Valdecoxib was used as an internal standard (IS) [24].

Preparation of internal standard

Accurately weighed 10 mg of IS was transferred into 100 ml volumetric flask containing 10 ml of acetonitrile (HPLC grade), which was sonicated for about 2 min. The volume was made up to the mark with acetonitrile. The stock solution was further diluted with mobile phase to give the final concentration of 1000ng/ml of each vial.

Detection of peak

From the various trails of IS and drug concentration, the optimized concentrations of IS 1000 ng/ml and pure drug (Eplerenone) 100 ng/ml was dissolved in a similar solvent and injected into HPLC to elute the peak. The prepared samples were evaluated for identification of individual peaks of ACN, IS and Eplerenone. The retention time of Eplerenone was found to be 4.5 min. Number of theoretical plates are 6087 with tailing factor 1.92 [25].

Construction of standard graph

Standard solution of Eplerenone at different concentrations were prepared and their peak area was determined by HPLC, calibration

curve was built by plotting the concentrations levels of drug ranges from 10-60 µg/ml in x-axis *versus* the corresponding peak area in y-axis, as shown in table 1. The correlation coefficient of Eplerenone is 0.9994 and hence the curve is said to be linear with y= 436607x+451518 as shown in fig. 1.

RESULTS AND DISCUSSION

The maximum absorbance was observed at 240 nm; further analysis was carried out at this corresponding nm.

Table 1: Calibration data of the standard graph

S. No.	Concentration (µg/ml)	Peak area
1	10	899056±145.34
2	20	1332754±207.94
3	30	1745378±176.87
4	40	2167349±6374.32
5	50	2656889±321.01
6	60	3076431±31.37

*All the values were expressed in (n=3) mean±SD

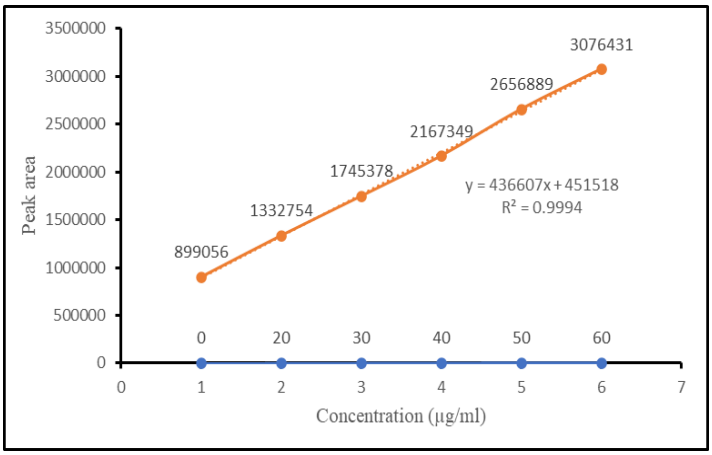

Fig. 1: Standard calibration curve of EP

Eplerenone floating microspheres were prepared using the solvent evaporation method. In the preparation of microspheres, ethyl cellulose has been used as a matrix material to achieve sustained release of drugs due to its chemical stability, water-insolubility, flexibility, and low price. Solvent diffusion method is also capable to improve loading efficiency of a water-soluble drug and modulate release profile [26].

The percentage yield was found to be in the range of 89.56±0.74% to 79.62±0.48% for microspheres of Eplerenone. Entrapment efficiency was 55.34% to 89.71%; buoyancy percentage was 99.16±0.83 to 89.84±0.21; size range was between 290.71 µm to 233.58 µm, zeta potential was found to be at-21.5 mV, results of SEM revealed that the microspheres using EC alone were discrete, rough outer surfaces, which might be due to cross-linking of the polymer with PVA, a piercing endothermic peak at 236.95 °C was observed in the finalized formulation corresponding to the melting point observed in differential scanning calorimetry of pure drug was 236.95 °C, the value of the relative degree of crystalline was 1.0. So X-ray diffraction analysis revealed that there was no change in the crystalline nature of the drug and found to stay stable in the final formulation [27]. Eplerenone have appeared in the samples without a noticeable change in their positions, indicating no chemical interaction between Eplerenone and polymers; optimized formulation showed a maximum release of 98.47 % of the drug up to 24 h, a stability study was conducted at 25 °C±2 °C and 60±2% relative humidity for 6 mo, the results showed that the formulation was stable.

Barium Sulphate helps to uncover dosage form in the GIT by which one can predict and correlate the gastric emptying time and the passage of dosage form. In gastro-scope and ultrasonography studies can be included in the *in vivo* evaluation of gastroretentive drug delivery systems [28]. The scintigraphs showed that all the formulations were intact in the physiological environment of stomach as depicted in fig. 2. Gamma scintigraphy study was performed to evaluate qualitative gastro retention following oral administration of barium sulphate formulations and to support quantitative pharmacokinetic outcomes. The images were captured at 1, 2, 4, 8 and 12 h post-administration. The rabbits showed an accumulation of microspheres at 1 h, which reached to maximum at 24 h showing prominent retention in gastric environment [29].

The correlation coefficient of Eplerenone is 0.9994 and hence the calibration curve was said to be linear. The formulation development and *in vivo* studies of Eplerenone shows better pharmacokinetic properties for its sustained action. For comparison, a formulated floating tablet of Eplerenone was used in the study. In the present studies peak plasma concentration of (Cmax) floating microspheres was found to be 1.17µg/ml against 1.29 µg/ml for floating microspheres of Eplerenone, time to reach peak plasma concentration (t-max) for floating tablet was 1.5 h, against 6 h for formulated Eplerenone floating microsphere. The area under the curve AUC for formulated floating tablet was 9.69 µg/ml, whereas for Eplerenone floating microspheres, it was 16.28 µg/ml, formulated floating tablet absorption rate constant Ka was 1.61 h and for optimized formulation, it was 0.5 h^{-1}. Elimination rate constant was 0.112 h and 0.053 h for formulated floating tablets and optimized formulation, respectively. Elimination half-life was found to be 6.2 hand 12.98 h for formulated floating tablets and optimized formulation, respectively. The results of Peak are and concentrations were depicted in table 2 and the results of pharmacokinetics analysis were depicted in table 3 and fig. 3.

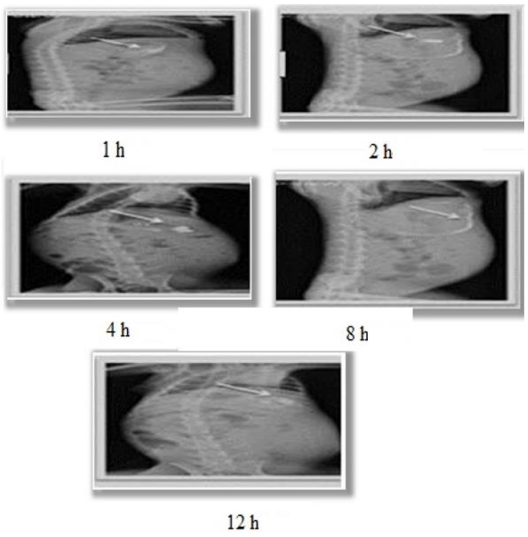

Fig. 2: Scintigraphy of floating microspheres

Table 2: Eplerenone plasma concentration in rabbits

Time (h)	Peak area		Concentration (µg/ml)	
	Formulated floating tablets	Floating microspheres	Formulated floating tablets	Floating microspheres
0	0	0	0	0
1	519562.3±43660	244499.9±35290	1.19±0.21	0.56±0.05
2	480267.7±82185	488999.8±55628	1.10±0.42	1.12±0.06
4	358017.7±21537	825187.2±24309	0.82±0.53	1.89±0.65
6	336187.4±27266	1117714±41266	0.77±0.64	2.56±0.34
8	283794.6±24309	777160.5±112618	0.65±0.22	1.78±0.37
10	240133.9±25582	318723.1±99210	0.55±0.54	0.73±0.78
12	43660.7±6669	52392.84±4366	0.10±0.32	0.12±0.30

*All the values were expressed in (n=3) mean±SD

Table 3: Results of the pharmacokinetic analysis

S. No.	Pharmacokinetic parameters	Units	Formulated floating tablets	Floating microsphere
1	Peak Plasma Concentration (Cmax)	(µg/ml)	1.17±0.42	1.29±0.21
2	Time to reach peak plasma concentration (Tmax)	(h)	1.5±0.32	6.0±0.30
3	Area under the curve (AUC_{0-24})	µg x h/ml	9.69±0.53	16.28±0.22
4	Lag time (T_{lag})	(min)	0	15±0.26
5	Absorption rate constant (Ka)	(h)	1.61±0.34	0.5±0.65
6	Elimination half-life(T½)	(h)	6.2±0.35	12.98±0.5
7	Elimination rate constant (Ke)	(h)	0.112±0.95	0.053±0.42

All the values were expressed in (n=3) mean±SD

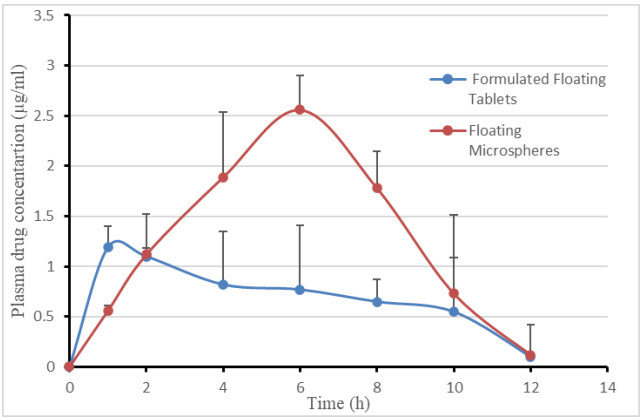

Fig. 3: Plasma level vs. time curve after oral administration of eplerenone-formulated floating tablets and floating microspheres; all the values were expressed in (n=3) mean±SD

The comparison of these data shows that the Cmax was not much valid, but AUC was increased to about 1.68 times in case of floating microspheres, absorption rate constant was found to be decrease 3.22 times when related to the floating microspheres, whereas Ke was found to be decrease 2.11 times when equated to floating microspheres, elimination half-life was increased by almost about two times [30]. In this study it was observed that absorption rate constant and elimination rate constant were appreciable decreased by encapsulation of Eplerenone into floating microspheres it was desirable for sustained the release of Eplerenone from the formulation is achieved in order to prolong the drug action, minimizing the frequency of drug administration and reduce sudden drug concentration fluctuations in the blood [31]. The *in vivo* study of desired formulation by software shows their ability to modify the pharmacokinetic behavior of the market formulation. These results clearly indicate that the sustained action of Eplerenone from this formulation was achieved for the purpose of research work.

CONCLUSION

Incorporation of Eplerenone in the microsphere proved to be an effective method to achieve the desired release of drug molecules and dosage form buoyancy. The designed system has excellent buoyance character which is suitable for drug release pattern; this dosage form has proven added advantage of increased bioavailability of Eplerenone. The developed floating microsphere of Eplerenone are found to be more effective when compared with floating tablets, thus prolonged action is needed for the particular category of the drugs (anti-hypertensive). The microsphere could be filled into capsules or formulated as oral suspensions for reconstitution for delivery of the drug.

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AUTHORS CONTRIBUTIONS

All authors are contributed equally.

CONFLICT OF INTERESTS

Declared none

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