Biological Activity of Rats Treated with Riboflavin in Polymeric PLGA as Injectable Form

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النشاط البيولوجي في الجرذان المعاملة بالريبوفلافين المحقونة بالبوليمر (بي ال جي اي)

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Abstract

The current study aims to optimize a fast, reproducible, reliable, and effective technique for estimating the biological activity of Vitamin B2 in injectable dose form using reversed phase high performance liquid chromatography (RP-HPLC), a biodegradable polymer that has been approved as an active ingredient for parenteral administration, the vitamin loaded in PLGA (Poly (lactic-co-glycolic acid) 50:50 type, and the injection volume was 1mg of prepare. Rats, both male and female, were employed as the animals. Individually, the duration time of the hosted animal reached 40 days, and the activity of vitamin B2 was recorded in rapid hours through six weeks by HPLC device of plasma sample, the results indicate that all formulations batch response continuously through forty days, with the best one detected according to drug bioavailability with inside the permitted range, the best performance mentioned at Fabs reached 108 percent.

Keywords: Micro-particles, PLGA, HPLC, Pharmacokinetics, Vitamin B2, Riboflavin, Drug Delivery System, In vivo Release.

الملخص

هدفت الدراسة الحالية الى تحسين تقنية سريعة وقابلة للتكرار وموثوقة وفعالة لتقدير النشاط البيولوجي لفيتامين بي 2 في حقن جرعة باستخدام تقنية كروموتوكرافيا سائلة عكسية عالية الاداء , تمت الموافقة على بوليمر كمكون نشط و قابلة داخلية, حيث تم تحميل الفيتامين على نوع بوليمر حامض اللاكتيك مطعم في بوليمر حامض الكلايكولك بنسبة 50:50 , وتم حقن حجم 1ملغم من البوليمر المحضر. في الحيوانات التي هيئت للحقن وهي الجرذان بكلا الجنسين المؤنث والمذكر. بشكل فردي ، وصلت المدة الزمنية للحيوان المستضيف إلى 40 يومًا, وسُجل نشاط فيتامين بي 2 في ساعات سريعة خلال ستة اسابيع بواسطة جهاز كروموتوغرافيا سائلة عكسية عالية الاداء لعينة البلازما. تشير النتائج إلى أن جميع التركيبات تستجيب بشكل مستمر خلال أربعين يومًا ، مع اكتشاف أفضلها وفقًا للتوافر الحيوي للدواء داخل النطاق المسموح به, بلغ افضل اداء مسجل في فابس الى نسبة 108.

الكلمات المفتاحية: الصيدلة الحركية, فيتامين بي2, الرايبوفلافين, نظام توصيل الدواء, اطلاق سراح داخلي و بي ال حي اي.

1. Introduction

Vitamin B2, often known as riboflavin, is one of the eight B Vitamins that are required for human health to function properly. It can be found in a variety of foods, including grains, vegetables, and dairy products (Ashoori and Saedisomeolia 2014). It is essential for the digestion of food components, the absorption of other nutrients, and the maintenance of tissues (Eussen *et al.*, 2011). B2 is a water-soluble vitamin; however, all vitamins, depending on their type, are either water- or fat-soluble (Chowdhury 1978). Individuals must consume Vitamin B2 on a regular basis since the body can store only a small amount of it and because supplies decrease quickly when not ingested (Aykroyd and Roscoe 1929), Riboflavin is found in naturally occurring forms in some foods, is added to others, and can be obtained through dietary supplements (Zylberman *et al.,* 2006)4-dihydroxy-2-butanone 4-phosphate with 5-amino-6-ribitylamino-2,4 (1H,3H. Riboflavin (Vitamin B2) is synthesized by condensing 3,4-dihydroxy- 2-butanone 4-phosphate with 5-amino - 6-ribitylamino- 2,4 (1H,3H) 6,7-dimethyl-8-ribityllumazine synthase catalyzes the formation of pyrimidinedione (lumazine synthase) (Jiang *et al.,* 2005)much progress has been made towards the clinical use of antigen-loaded microspheres. Poly(lactide-co- glycolic acids, there is two divisions of PLGA is a biodegradable polymer that has been certified as an active ingredient for parenteral administration by the Food and Drug Administration and the European Medicines Agency (Aguillón *et al.,* 2004). It is widely utilized in the pharmaceutical industry; Various medicinal applications have relied on this well-established medication delivery

mechanism for years (Chereddy *et al.,* 2014). In addition to medications such as antibiotics, anti-inflammatory pharmaceuticals, proteins/peptides, and nucleic acids which target different phases/signaling cycles of healing process, it is also mentioned that the cumulative therapeutic value of PLGA and a overloaded medication on injuries therapeutic are beneficial (O'Neill *et al.,* 2015).Therefore, the current study aims to optimize a fast, reproducible, reliable, and effective technique for estimating the biological activity of Vitamin B2 in injectable dose form using reversed phase high performance liquid chromatography (RP-HPLC),

2. Materials and Methods

2.1 Experimental Animals

The study's animal models were Sprague Dawley rats, both males and females, which were employed in the experiment. All protocols were approved by the University of Kentucky's Institutional Animal Care and Use Committee (IACUC), and the animals were housed in facilities recognized by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The animals stayed divided into four groups of eight each for testing, with one animal left untreated as a control. The dosing technique and findings showed that the average outcomes for the four formulations were obtained, as well as their standard deviations (standard error of the mean). During the course of these investigations, a total of 28 rats were administered with loaded vitamin B2 microspheres that were implanted in the subcutaneous area on the dorsal superficial under isoflurane anesthesia, which allowed for pharmacokinetic evaluations of plasma concentration levels over time.

2.2 Pharmacokinetic Study

The analysis was carried out using a high-performance liquid chromatography system with UV detection at 215 nm. Blood samples weighing 0.5 mL were taken at each of the three time points. The blood was centrifuged for 30 minutes, with 200 mL of serum remaining to be frozen at -80°C until analysis was performed (Fernandes *et al.,* 2007), (Elbarbry, Wilby, and Alcorn 2006) and (Niu *et al.*, 2012).

The amounts of Vitamin B2 in the serum of each animal were determined in duplicate at each of the time points. A standard solution of Vitamin B2 was prepared in normal rat serum (with a concentration range of 1.25 - 50 ng/ml). When the standard solutions were prepared, they were extracted using a process that was the same as that used for the research samples. They were included in each run to provide both the standard curve and retention time for the drug under investigation. Vitamin B2 had a retention duration of around 25 minutes when tested. It was established that the final in vivo serum concentration profile for each rat was obtained by graphing serum concentration against time for all animals (Niu *et al.,* 2012) ,(Hasimoglu and Ghodke 2018) and (Jakobsen 2008).

2.3 Behavioral Studies

Under the influence of isoflurane anesthesia, microspheres were implanted in the subcutaneous area on the dorsal surface. In order to look for indicators of initial high drug release during this period, daily qualitative evaluations of the rats being performed for five days immediately following transplantation in order to make it look for indications of increased initial drug release throughout this period.

3. Results and Discussion

3.1 Pharmacokinetic Studies Results

The In vivo pharmacokinetic characteristics of PLGA micro-particles were examined in rats in order to evaluate the feasibility of developing a long-term, sterile drug delivery system for Vitamin B2 supplementation. Rats were given either EO sterilized PLGA-B2 or surface sterilized (betadine wash) PLGA 50:50-B2.

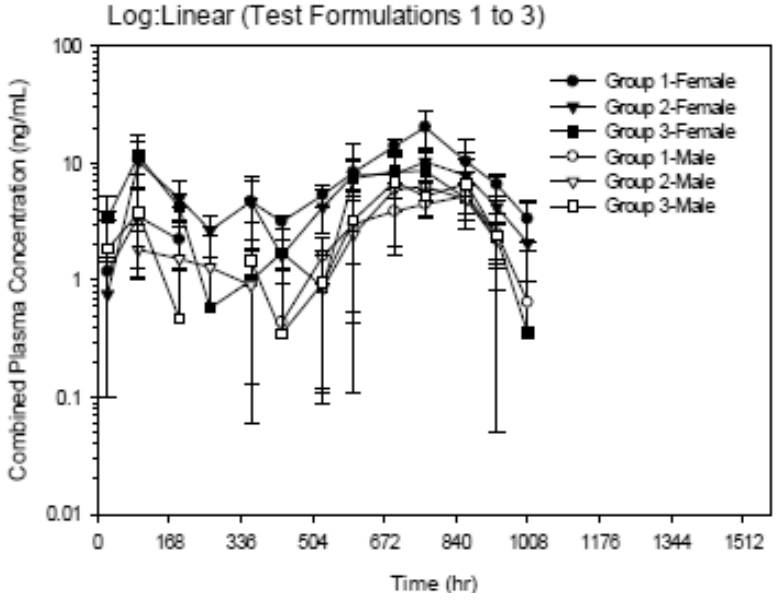
The In vivo onset was quick, and the serum concentration remained within the intended range of 2-15 ng/ml for a significant amount of the release interval, despite the fact that serum levels fluctuated over time. At 14 days, serum levels were roughly 7-10 ng/ml, and by 27 and 40 days, they had climbed to approximately 15-20 ng/ml (Figures 4 - 6). As a result, the microspheres continued to supply Vitamin B2 for at least 40 days.

Following sample collection, the plasma levels for each animal were evaluated for Vitamin B2. Figures (1 to 6) represent the In vivo release over the 30-day period for each of the formulation groups. These Figures use the average plasma level of Vitamin B2 (ng/mL) for each animal at each time-point pulled.

Due to the elimination of any remaining sterile microspheres from some of the rats, the presence of Vitamin B2 was determined by HPLC/UV spectroscopy in the remaining animals. Upon analyzing the amount of drug present in the extracted samples, it is observed that the percent drug load decreases over time, indicating that the drug is being released at a faster rate than the polymer is dissolving. Approximately 25 wt. % of the micro-particles removed on day 14 have a load of approximately 1 to 1 wt. percent on day 15, 15 % on day 27, and 0 to 1 wt. % on day 30. Microspheres removed at 40 days have a drug load of about 0 wt. percent, indicating that the drug has been

completely released before this time point. There was no evidence of local tissue response, fibrosis, or erosion upon the micro-particles of Vitamin B2 release in the body, indicating that the micro-particles of B2 release were biocompatible locally throughout the implementation period at all-time points. All figure test formulation represents the bioavailability in different ranges.

The animal analyzing study showed five test formulations for five different patches, test of formulation patches included using eight of males and females rats with number as it is and after single subcutaneous injection of 1mg/animal of prepared vehicle of Vitamin B2 to male rats, as shown in Figure (1).

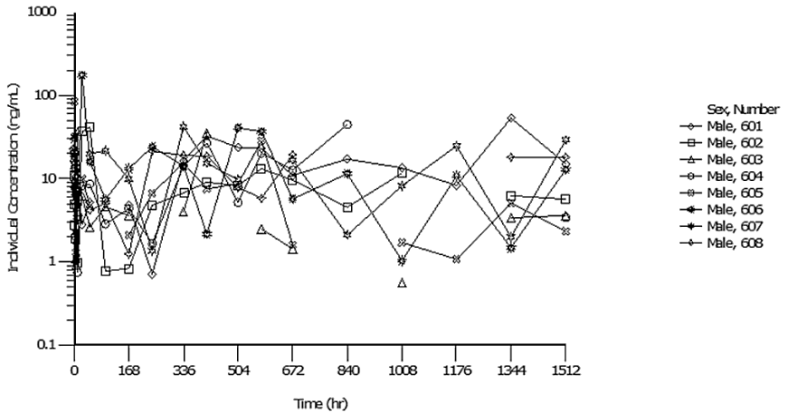


Figure(1) Individual and Mean Vitamin B2 Cmax and AUC0-1512hr Values Test Formulations or Reference Product

All figures of analyzing the animal activity for Vitamin B2 will represent in logarithmic plot. The value of bioavailability acceptable rang (80 – 125) % (Tang *et al.,* 2014), Table 1 summarized the bioavailability for all formulations patches.

Table(1) Bioavailability for tested formulation in rats' bioactivities

FC	F%
1	35%
2	56%
3	108%
4	144%



Figure(2) Individual Vitamin B2 Plasma Concentration-Time Profiles Formulation 1

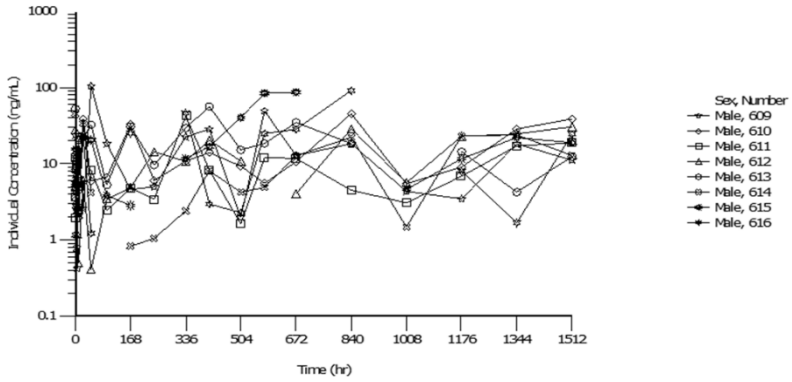


Figure (3) Individual Vitamin B2 Plasma Concentration-Time Profiles Test Formulation 2

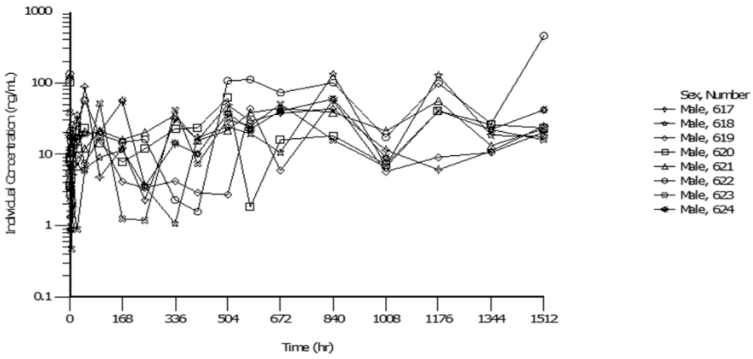


Figure (4) Individual Vitamin B2 Plasma Concentration-Time Profiles Test Formulation 3

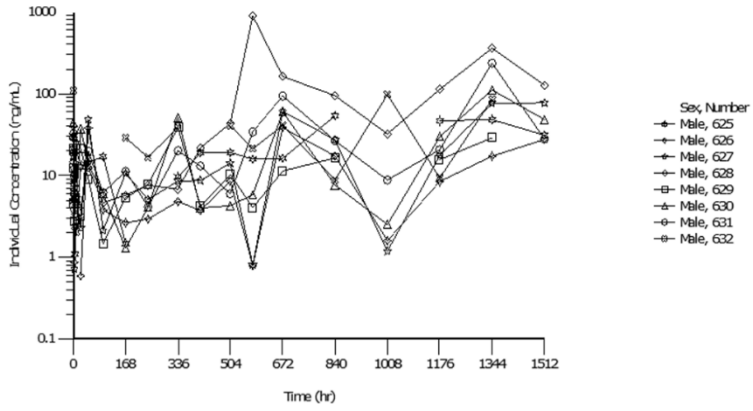


Figure (5) Individual Vitamin B2 Plasma Concentration-Time Profiles Test Formulation 4

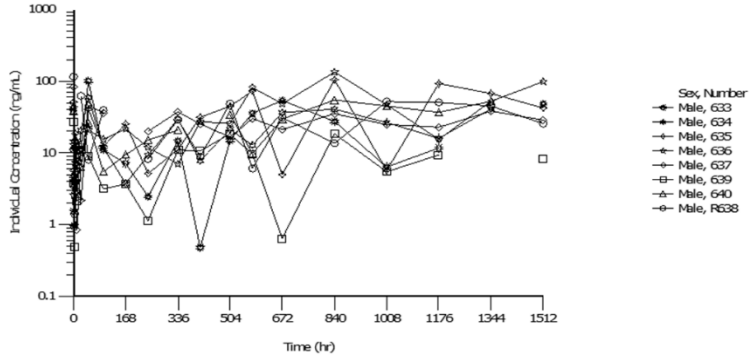


Figure (6) Individual Vitamin B2 Plasma Concentration-Time Test Formulation 5

4. conclusion

The industrialized RP-HPLC method offers a convenient and well-organized method for the quantitative estimation of a new modification of Vitamin B2 drug and pharmaceutical dosage form. This method has various advantages simple, precise, quick analysis time 40 days, The animal study results showed that the activity of all formulation tests to male rat all eight-male rate showed response to Vitamin B2 injection with different dose concentrations, formulation 1, 2, 3, 4 and 5) showed the ratio of area under the carve of product to the reference (35%, 56%, 108%, 144% and up to 150%) respectively and most appropriate dosage form with test formula number 4.

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