13M0330

Reverse engineering of the one-month Lupron Depot®

Keiji Hirota[1,†], Jia Zhou[1], Rose Ackermann[1], Yan Wang[3], Stephanie Choi[3], Anna Schwendeman[1] and Steven P. Schwendeman[1,2]

[1]Department of Pharmaceutical Sciences, The Biointerfaces Institute, University of Michigan; [2]Department of Biomedical Engineering, University of Michigan; [3]Office of Generic Drugs, U.S. Food and Drug Administration; [†]Current address: Production Engineering Department, Chugai Pharmaceutical Co., Ltd., Japan

2016 AAPS ANNUAL MEETING AND EXPOSITION
NOVEMBER 13–17, 2016
COLORADO CONVENTION CENTER, DENVER

PURPOSE

The 1-month Lupron Depot® (LD) is a brand mark poly(lactic-co-glycolic acid) (PLGA) microsphere product upon which modern long-acting release products are often compared. Launched in the US in 1989 the LD encapsulates and slowly releases leuprolide acetate to reduce injection frequency relative to daily injections of soluble peptide. Despite expiration of patent coverage, there exists no generic for LD on the US market. To help enable generic development we sought to determine the detailed composition and develop the related assays to accomplish this task. The specific components analyzed included: leuprolide content; gelatin type and content; PLGA content, lactic acid/glycolic acid (LA/GA) ratio and molecular weight distribution, and mannitol content; pH of diluent and water content in diluent.

METHODS

- Chamber 1 (Powder)
  - Dissolved in methylene chloride and extracted by phosphate buffer → UPLC → Leuprolide loading
  - Colorimetric assay kit → Mannitol content
  - Amino acid analysis → Gelatin content
  - Remove mannitol by water
    - qNMR → PLGA content and LA/GA ratio
    - GPC → PLGA molecular weight
    - Dissolved in methylene chloride and gelatin was extracted by warm water; purification → Gelatin typing by ion exchange chromatography
- Chamber 2 (Diluent)
  - Colorimetric assay kit → Mannitol content
  - Fluorescence reaction → Tween 80 content
  - Gravimetry → Water content

RESULTS

Leuprolide content in three different batches determined by single extraction was 8.31 ± 0.05 wt% in the LD microspheres, showing close values relative to the official content (8.5 wt%) [1]. Multiple extractions yielded slightly higher loading values. Several other components were also determined to be very similar to the literature reported values [1-3] (Figure 1) . As determined by NMR, the PLGA in LD displayed an 86.5 ± 0.3 wt% content compared with 88.3 wt% (without mannitol). Similarly, the content of D-mannitol in the LD was measured to be 15.6 ± 0.4 wt%, which is close to the official content of 15.0 wt% [1]. Preliminary analysis of gelatin content by amino acid analysis indicated 1.52 wt% compared with 1.5 wt% [1]. Gelatin extracted from the LD displayed a pronounced basic peak as the type B gelatin, indicating that the gelatin used for the LD formulation is type B (Figure 2). The 1-month LD has been reported [1-3] to be composed of PLGA with an LA/GA ratio, 75:25; Mw, 12.1 to 14 kDa; and a ratio of Mw to Mn (PDI), 1.6 to 1.7. The PLGA in LD displayed 74.3 ± 0.1/25.7 ± 0.1 LA/GA ratio, 13.04 ± 0.06 kDa for Mw, 8.67 ± 0.05 kDa for Mn, and ~1.5 for PDI which are very close to previously reported ones mentioned above.

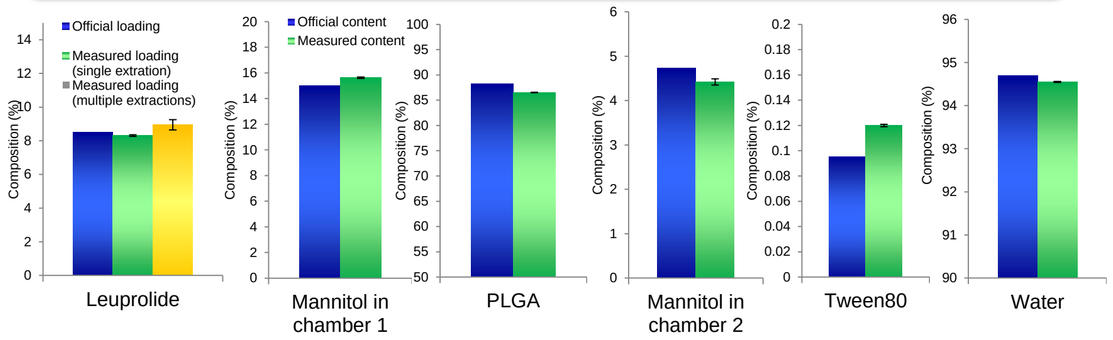

Figure 1. Comparison of official content and measured content of components in chamber 1 and chamber 2 of LD. Columns represent mean (%) ± SEM (n=3).

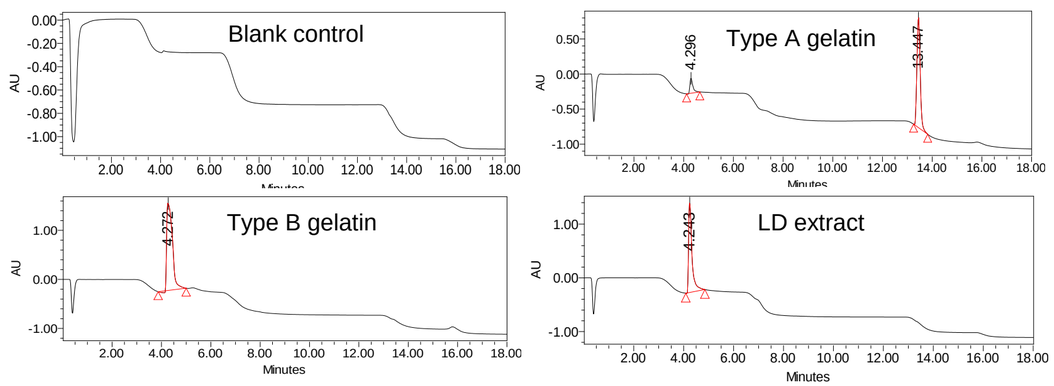

Figure 2. Representative ion exchange chromatograms of blank control, type A gelatin, type B gelatin and gelatin extracted from LD. Pure type A and B gelatins have a major peak at a retention time around 13.5 min and around 4.2 min, respectively. Gelatin used for Lupron Depot formulation was identified as type B.

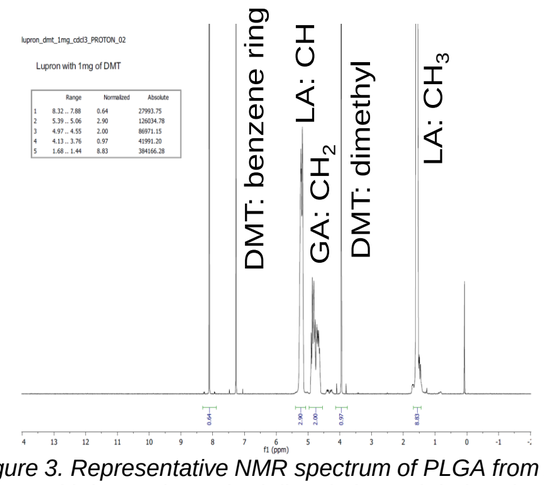

Figure 3. Representative NMR spectrum of PLGA from Lupron Depot with internal standard dimethyl terephthalate (DMT).

CONCLUSIONS

Analytical methods for analyzing the specific components of the 1-month Lupron Depot® have been developed and the ingredients have been identified and quantified. The results match well the official content or reported values. The analysis described here could be useful for further development of generic leuprolide microspheres, and for composition assay assessment of new and existing microsphere formulations.

FUNDING/ REFERENCE

This research was funded by FDA contract HHSF223201510170C A0001 BAA.

Disclaimer:
This poster reflects the views of the author and should not be construed to represent FDA's views or policies.

Reference:

[1] Takeda Pharmaceutical Company. Lupron Depot® package insert.
[2] Ogawa, Y.; Yamamoto, M.; Takada, S.; Okada, H.; Shimamoto, T. Chem. Pharm. Bull. (Tokyo) 1988, 36, 1502.
[3] Okada, H.; Inoue, Y.; Heya, T.; Ueno, H.; Ogawa, Y.; Toguchi, H. Pharm Res 1991, 8, 787.