EXTRACTION FROM AND ASSAY OF BACTERIAL ENDOTOXINS IN OIL PARENTERAL PREPARATIONS: ESTRADIOL® (Oestradiol Dipropionate) AND LUSTESTROL® (Progesterone + Oestradiol Benzoate) INJECTION

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Introduction

The compendial requirements are provided for a large number of medicinal products (EP, BP, USP and other relevant pharmacopoeias) as well as manufacturer’s requirements for bacterial endotoxins limit concentration (ELC) in parenteral products. However, there are products that are not covered by the requirements for ELC; moreover, they may be in such form (oil solution) that hinders either the in vivo pyrogen testing with rabbits or the assessment of bacterial endotoxins using in vitro qualitative or semi-qualitative (gel clot) method or a quantitative one (kinetic turbidimetric or kinetic chromogenic method). For testing bacterial endotoxins in oil preparations it is first necessary to extract the bacterial endotoxins from the oil solution. If the requirement for ELC in the tested preparation is unavailable, that ELC needs to be established (calculated). This paper presents the procedure for extraction and establishment (calculation) of the ELC in oil parenteral products: ESTRADIOL® Injection (10 mg of Oestradiol Dipropionate/ml), LUSTESTROL® Injection (20 mg of Progesterone + 2 mg of Oestradiol Benzoate/ml).

Methods and results

Extraction of bacterial endotoxins from oil solutions (recommended by Charles River Endosafe). 1) Take 0.5 ml of oil solution and transfer into a sterile test tube. 2) Add 5 ml of LAL water reagent. Combine the oil solution and LAL water reagent as follows: a) shake on the vortex 5 x 30 seconds at 30-second intervals, or b) shake on the vortex alternately for 1 hour, or c) centrifuge, as required, at 2300 rpm for 10 minutes. 3) Following vortex allow the phases to separate. 4) Use aqueous phase for further vortexing. 5) The aqueous phase is equivalent to 1:10 dilution of the product. The extraction procedure is based on the assumption that bacterial endotoxins will come over to the aqueous phase.

ESTRADIOL® Inj. Starting from the fact that the highest daily i.m. therapeutic dose of oestradiol dipropionate for humans is 10 mg, the ELC in LUSTESTROL® Inj. is calculated. The calculated ELC value for oestradiol dipropionate is 34.97 EU/mg.
The mathematically determined value of ELC for oestradiol dipropionate was verified by numerous experiments using Limulus Amebocyte Lysate of different sensitivity (LAL test, gel-clot method). The obtained results showed that the content of bacterial endotoxins in ESTRADIOL® Inj. was lower than the calculated value of ELC. The quantitative turbidimetric method of LAL test showed that the content of bacterial endotoxins in ESTRADIOL® Inj. was 1.4 EU/mg (spike recovery (%): 95.0, 117.0 and 113.0, for 3 different batches, must be between 50 and 200%).

**LUTESTROL® inj.** Starting from the fact that the highest daily i.m. therapeutic dose of progesterone for humans is 200 mg [1], the ELC in LUTESTROL® Inj. is calculated. The calculated ELC progesterone value is 1.75 EU/mg has also been challenged by numerous experiments using Limulus Amebocyte Lysate of different sensitivity (LAL test, gel-clot method). The obtained results showed that the bacterial endotoxin content in LUTESTROL® Inj was lower than the calculated ELC value. The quantitative turbidimetric method of the LAL test showed that the bacterial endotoxin content in LUTESTROL® Inj was 0.01 EU/mg (spike recovery (%): 95.6, 91.1 and 108.5, for 3 different batches, must be between 50 and 200%).

**Check of the effectiveness of bacterial endotoxin extraction from oil solutions with standard addition.** Standard addition: CSE, E. Coli 055: B5, 500 ng, Lot: EM84632, 6.000 EU/bottle, Charles River Endosafe.

**ESTRADIOL® Inj.** Based on the assessed ELC value of oestradiol dipropionate (34.97 EU/mg) and the content of oestradiol dipropionate in the tested product (10 mg/ml), the ELC in the tested product was made (349.7 EU/ml of emulsion) and extracted as described.

Four weighed concentrations of bacterial endotoxins were: 35; 3.5; 0.35 and 0.035 EU/ml and the calculated ones were: 26; 3.3; 0.27 and 0.025 EU/ml (coef. cor. r = 0.9993, must be ≥ 0.98).

**LUTESTROL® inj.** Based on the assessed ELC value of progesterone (1.75 EU/mg) and the content of progesterone in the tested product (20 mg/ml), the ELC in the tested product was made (35 EU/ml of emulsion) and extracted as described.

Four weighed concentrations of bacterial endotoxins were: 3.5; 0.35; 0.035 and 0.0035 EU/ml and the calculated ones were: 2.1; 0.22; 0.02 and 0.006 EU/ml (coef. cor. r = 0.9909, must be ≥ 0.98).

**Conclusion**

1) Extraction of bacterial endotoxins from oil solution is achieved by vortexing the oil-water emulsion (1:10 ratio), 5 x 30 seconds at 30-second intervals.

2) The endotoxin limit concentration for oestradiol dipropionate is 34.97 EU/mg.

3) The endotoxin limit concentration for progesterone is 1.75 EU/mg.

**References**