EXTRACTION FROM AND ASSAY OF BACTERIAL ENDOTOXINS IN OIL PARENTERAL PREPARATIONS. II: PROGESTERON DEPO® (hydroxyprogesterone caproate) and TESTOSTERON DEPO® (testosterone enanthate) INJECTION

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Introduction
For testing bacterial endotoxins in oil preparations it is first necessary to extract the bacterial endotoxins from the oil solution. If the requirement for endotoxin limit concentration (ELC) in the tested preparation is unavailable, that ELC needs to be established (calculated).

In previously paper [1] has been presented the procedure for extraction and establishment (calculation) of the ELC in oil parenteral products: ESTRADIOL® Injection (10 mg of oestradiol dipropionate/ml) and LUTESTROL® Injection (20 mg of progesterone + 2 mg of oestradiol benzoate/ml).

This paper presents the procedure for extraction of the bacterial endotoxins in oil parenteral products: PROGESTERON DEPO® inj. (250 mg of hydroxyprogesterone caproate /ml), and TESTOSTERON DEPO® inj. (250 mg of testosterone enanthate /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.

PROGESTERON DEPO® Inj. Starting from the fact that the highest daily i.m. therapeutic dose of hydroxyprogesterone caproate for humans is 500 mg [2], using the formula for calculating ELC [3], the ELC in PROGESTERON DEPO® Inj. is calculated.

The calculated ELC value for hydroxyprogesterone caproate is 0.70 EU/mg. The mathematically determined value of ELC for hydroxyprogesterone caproate 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 PROGESTERON DEPO® Inj. was lower than the calculated value of ELC. The quantitative turbidimetric method of the LAL test showed that the content of bacterial endotoxins in PROGESTERON DEPO® Inj. was 0.054 EU/mg (targeted positive control of the sample (%): 111.0, 106.4 and 104.0, for 3 different batches, must be between 50 and 200%).

TESTOSTERON DEPO® Inj. Starting from the fact that the highest daily i.m. therapeutic dose of testosterone enanthate for humans is 400 mg [4], the ELC in TESTOSTERON DEPO® Inj. is calculated. The calculated ELC testosterone enanthate value is 0.88 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 TESTOSTERON DEPO® Inj. was lower than the calculated ELC value. The quantitative turbidimetric method of the LAL test showed that the bacterial endotoxin content in TESTOSTERON DEPO®
Inj was 0.0044 EU/mg (target positive control of the sample (%): 99.6, 154.7 and 185.4, 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). Standard curves: 5; 0.5; 0.05 and 0.005 EU/ml.

PROGESTERONE DEPO® Inj. Based on the assessed ELC value of hydroxyprogesterone caproate (0.70 EU/mg) and the content of hydroxyprogesterone caproate in the tested product (250 mg/ml), the ELC in the tested product was made (175 EU/ml of emulsion) and extracted as described. Four weighed concentrations of bacterial endotoxins were: 17.5; 1.75; 0.175 and 0.0175 EU/ml and the calculated ones were: 18.8; 1.56; 0.134 and 0.009 EU/ml (coefficient of correlation r = 0.9998, must be ≥ 0.98). (Fig.1.)

TESTOSTERONE DEPO® Inj. Based on the assessed ELC value of testosterone enanthate (0.88 EU/mg) and the content of testosterone enanthate in the tested product (250mg/ml), the ELC in the tested product was made (220 EU/ml of emulsion) and extracted as described. Four weighed concentrations of bacterial endotoxins were: 22; 2.2; 0.22 and 0.022 EU/ml and the calculated ones were: 20.7; 2.21; 0.23 and 0.019 EU/ml (coefficient of correlation r = 0.9997, must be ≥ 0.98). (Fig.2)

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 hydroxyprogesterone caproate is 0.70 EU/mg.
3) The endotoxin limit concentration for testosterone enanthate is 0.88 EU/mg.

References