CALCULATION OF BACTERIAL ENDOTOXIN LIMIT CONCENTRATION IN INJECTABLE PRODUCTS: DIKLOFEN® (diclofenac sodium) AND NOVALGETOL® (metamizole sodium) INJ.

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Introduction

By their chemical character, bacterial endotoxins are lipopolysaccharides found in Gram-negative bacteria. Throughout their lives, people are exposed to minute quantities of endotoxins that released after bacterial death (mostly Escherichia coli Berget) in the gastrointestinal tract. This small endotoxin quantity induces the production of moderate quantity of antibodies to oppose endotoxins. However, in times of gastrointestinal crises (e.g. in case of ileus when gangrene occurs causing a sudden increase of the number of bacteria), a large quantity of endotoxins may enter the circulation leading to anaphylactic response resulting in a severe shock (endotoxin shock).

Due to the consequences to human health that could result from bacterial endotoxin exposure, one of the compendial requirements for granting authorisation to injectable medicinal products is the test for bacterial endotoxins that may be present in these products (determination of bacterial endotoxin content).

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 the products. However, some products are not covered by the compendial bacterial ELC requirements. To enable the assessment of bacterial content in these products, it is necessary to evaluate (calculate) the bacterial ELC in these products.

This paper presents the determination (calculation) of the bacterial ELC in DIKLOFEN® Inj. (75 mg of diclofenac sodium/3 ml) and NOVALGETOL® Inj. (2.5 g of metamizole sodium/5 ml).

Methods and results

DIKLOFEN® Inj. (diclofenac sodium)
The bacterial ELC in DIKLOFEN Inj. was evaluated by two methods using the following data and formulae.

1st Method
1) The maximum daily therapeutic dose of any diclofenac sodium containing formula in man is 150 mg [1]
2) ELC of diclofenac sodium was determined using the formula ELC = K / M, where ELC is endotoxin limit concentration, K is maximum acceptable bacterial endotoxin content skg body mass (this is a constant; for diclofenac sodium, it is 2.143 mgskg), which was 2.33 EUsmg.

2nd Method
1) MVC of diclofenac sodium was determined for different sensitivity Limulus amebocyte lysate using the formula MVC = ( λ * M ) / K, where MVC is minimum valid concentration of the tested sample, λ is the sensitivity of the tested Limulus amebocyte lysate used (in EU/ml), and the meanings of M and K parameters have been shown previously.
2) MVD of diclofenac sodium was determined for different sensitivity Limulus amebocyte lysate using the formula MVD = C / MVC, where MVD is the maximum valid dilution of the tested sample, C is the concentration of the tested sample (in mg/ml) (25 mg/ml for diclofenac sodium), and using the known MVC value [2].
3) Using a different mathematical form of the formula for MVD assessment, i.e. MVD=(ELC * C) / λ, and knowing the value for MVD, C concentration of the tested sample (in mg/ml) (C for diclofenac sodium is 25 mg/ml) we obtained the identical value for ELC of diclofenac sodium (2.33 EU/mg).

The mathematically determined value of diclofenac sodium ELC was experimentally demonstrated using different sensitivity limulus amebocyte lysate (LAL test, gel-clot method). The obtained results showed that the content of bacterial endotoxins in DIKLOFEN® Inj. was lower than the calculated ELC value.
The quantitative turbidimetric method of the LAL test showed that the bacterial endotoxin content of DIKLOFEN® Inj. was lower than 0.015 EU/mg (spike recovery (%): 104.5, 105.1 and 111.5, 3 different series, must be between 50-200%).

**NOVALGETOL® Inj. (metamizole sodium)**

Taking into consideration that the maximum daily therapeutic i.m. dose of metamizole sodium in man is 7.5 g, the bacterial ELC in NOVALGETOL® Inj. was determined in the same way. The calculated ELC of metamizole sodium was 0.047 EU/mg, which was demonstrated by several retests using the limulus amebocyte lysate of different sensitivity (LAL test, gel-clot method). The obtained results showed that the content of bacterial endotoxins in NOVALGETOL® Inj. was lower than the calculated ELC value. The quantitative turbidimetric method of the LAL test showed that the bacterial endotoxin content of NOVALGETOL® Inj. was lower than 0.0046 EU/mg (spike recovery (%): 95.1, 95.1, 95.1 and 97.8, 4 different series, must be between 50-200%).

**Conclusion**

The endotoxin limit concentration for diclofenac sodium is 2.33 EU/mg. The endotoxin limit concentration for metamizole sodium is 0.047 EU/mg.

**References**
