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Development and Validation of a Method for Quantitative Determination of Innovative Antitumor Peptide Drugs in Order to Study their Pharmacokinetics

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1. Introduction

According to the World Health Organization (WHO), oncological diseases hold second place in the world in terms of mortality after cardiovascular diseases; and in the next few decades the number of cases will continue to increase [1]. Development of medicines based on modified endogenous peptides is one of the most relevant areas of modern medicine. The most interesting among them are antitumor peptide drugs, which are no less effective than chemotherapy with its pronounced side effects, or surgical interventions that often take a toll on patients [2]. When conducting studies of peptide preparations in biological fluids, the structure of the peptide molecule and its physicochemical properties are taken into account.

2. Materials and methods

We selected standard samples of the analyzed drugs goserelin acetate, busserelin acetate, octreotide acetate, triptorelin acetate, and dalargin acetate (internal standard) as subjects of the study, as well as samples of intact human blood plasma and mini-pig blood plasma. Reagents: acetonitrile (LC-MS grade, Biosolve, Israel), methanol (HPLC-grade, Panreac, Spain), formic acid (Extrapure, Sigma, USA), Milli-Q water. Equipment: Nexera high-performance liquid chromatograph (Shimadzu, Japan) equipped with a LCMS-8040 triple quadrupole mass-selective detector (Shimadzu, Japan).

The conditions of chromatographic separation were selected experimentally. Chromatographic separation was carried out on a Jupiter® 5 µm C18

50x4.6 mm 300Å column. As the mobile phase, a mixture of a 0.1% solution of formic acid in water (eluent A) and a 0.1% solution of formic acid in acetonitrile (eluent B) was used in the gradient elution mode at a rate of 1.2 ml/min. Detection was carried out in the monitoring multiple reactions (MRM) mode with positive electrospray ionization (ESI), 5 kV. Sample preparation was carried out using the method of solid-phase extraction (SPE) for quantitative determination in human blood plasma, and the method of protein precipitation with methanol for quantitative determination in animal blood plasma.

3. Results

The developed method for quantitative determination of antitumor peptide drugs was successfully validated according to the Drug Examination guide Volume I [3], as well as the guidelines of the FDA (US Food and Drug Administration) [4] and EMA (European Medicines Agency) [5] based on the following parameters: selectivity, linearity, matrix effect, correctness (inside the cycle and between cycles), precision (inside the cycle and between cycles), the lower limit of quantification, sample transfer, stability.

The analytical range of the method was 0.5-20.0 ng/ml for all analytes, with sample preparation using the method of solid-phase extraction. The correlation coefficients were: $r = 0.99834$ for goserelin, $r = 0.99855$ for busserelin, $r = 0.99791$ for triptorelin, and $r = 0.99841$ for octreotide. With sample preparation using the method of protein deposition, the analytical range of the method was 2.0–20.0 ng/ml for goserelin and 1.0–20.0 ng/ml for

buserelin and okreotide. The correlation coefficients were $r = 0.99774$ for goserelin, $r = 0.99821$ for buserelin, and $r = 0.99632$ for octreotide. The technique was not validated for triptorelin due to low sensitivity. The obtained values of RSD (relative standard deviation) and E (relative error) corresponded to the norms of the guidelines (no more than 20% for LLOQ and no more than 15% for other points).

4. Conclusions

Thus, in clinical and pharmacological studies of goserelin, buserelin, triptorelin and octreotide, solid-phase extraction is the optimal method for sample preparation of blood plasma due to the higher degree of analyte extraction. When conducting those types of pharmacokinetic studies where it is not necessary to determine the concentrations of the test substances below 1-5 ng/ml, such as preclinical tests, it is more appropriate to use protein precipitation with methanol for pre-

paring blood plasma samples. The obtained results confirm that the developed method can be used in clinical and pharmacological studies in patients with prostate cancer, as well as with the aim of assessing the bioequivalence of reproduced drugs.

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