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Method of high-performance liquid chromatography to determine features of feed

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Abstract.

Bacitracin is a part of an antibiotics group called polymyxin. It is characterized by cyclic peptide structures with the hydrophobic side chains used as a feed product for non-medical purposes. The most promising direction for optimization of zinc bacitracin (ZnB) qualitative and quantitative identification by means of high-performance liquid chromatography (HPLC) has not been studied at present. The aim of the research was to determine optimal modes and conditions for HPLC chromatography to identify zinc bacitracin antibiotic. The method is as follows: to extract bacitracin antibiotic residue according to the major active bacitracin A component from the analyzed sample: to make qualitative and quantitative identification by means of HPLC. HPLC system was established in order to separate zinc bacitracin antibiotic components analytically and preparatively. Peak moments have been registered without components decomposition under UV detection on Agilent 1260 Infinity and Spectra-Physics Spectra 100 chromatographs at a wavelength of 254 nm. Pre-column derivatization with o-phthaldialdehyde at the presence of 2-mercaptoethanol made it possible to identify the major component of the BC-A with the help of fluorescence detector Hitachi 850. 0.1 M disodium salt solution of ethylenediaminetetraacetic acid (EDTA) in the aqueous part of the mobile phase was selected as a mobile phase modifier to enhance the sensitivity of bacitracin peaks at low concentrations. The analysis of zinc bacitracin composition was made according to the research conducted with the help of HPLC methods by means of diode-array, UV, and fluorescence detectors. The possibility to increase the HPLC analysis sensitivity by adding an equimolar amount of EDTA in the mobile phase is shown. A method for ZnB identification by means of HPLC in the reversed phase of pre-column derivatization with the ortho-phthalic dialdehyde to enhance fluorescence was developed.

Key words: Antibiotics; zinc bacitracin; high-performance liquid chromatography

INTRODUCTION.

A world-wide steady trend of strict requirements to the quality of food products in order to exclude the antibiotics in animal livestock products sets the task to develop and introduce new, more effective, and sensitive methods of analysis [1,2,3].

Microbiological methods, enzyme immunodetection (ELISA), column and thin layer chromatography (TLC) have traditionally been used in reply to in the absence of the chemical methods for the bacitracin identification.

When ELISA is used, the microbiological research is conducted, as well as chromatography and TLC applied only a bacitracin qualitative detection is possible. These methods are also not specific enough and have a long period of analysis. Currently, a large number of additives (preservatives, dyes, flavor enhancers, etc.) is used in food products which can lead to false positive results for these studies.

In this regard, the necessity to develop highly sensitive and evidence-based methods in order to identify antibiotics contamination in the objects of veterinary surveillance is one of the main measures to ensure the safety of livestock products. There is a standard documentation on the bacitracin detection with the help of high-performance liquid chromatography based on the mass spectrometric detector in Russia. However, this method is applied only to meat and meat products. Procedures for sample preparation and identification of antibiotic residues in the feed are not provided in this document. Therefore, it cannot be used.

Thus, the development of the chromatographic method of bacitracin feed antibiotic identification by high-performance liquid chromatography (HPLC) is apparent [4,5].

Bacitracin is a mixture of polypeptide antibiotics, and each substance in the mixture has a complex structure. Bacitracin is a polypeptide antibiotic with various active components in the form of A_1, B_1, B_2, and B_3 having a major therapeutic effect, while bacitracin F is a decomposition product that shows neurotoxicity [6,7]. This means that the analysis of the bacitracin content in the samples is not an easy task. In addition, mixtures containing other substances of different origin and purpose are used as feed additives. It complicates further identification of bacitracin in feed and food [8,9].

The USA Pharmacopoeia monograph for zinc bacitracin includes a composite test that contains isocratic norms for HPLC method in which the content of bacitracin A should be at least 40%, and at least 70% of the amount for bacitracins A, B_1, B_2, B_3, and no more than 6% of bacitracin F product degradation [10].

Taking the Zn-BC physical and chemical properties into consideration, it can be assumed that its identification is possible by HPLC in case of the detector and proper selection of the absorption wavelength [11].

MATERIALS AND METHODS.

The standard zinc bacitracin containing 90% of the active substance (European Pharmacopoeia Reference Standard) was used in the experiments.

The following reagents were used to prepare for the mobile phase: acetonitrile, potassium monosodium phosphate, potassium disodium phosphate, monosodium phosphate, disodium phosphate, sodium tetraborate, methanol, ethyl

acetate, the disodium salt of ethylenediaminetetraacetic acid (EDTA), and phosphoric acid.

Standard solutions with 5–10 mg/kg concentrations were prepared in aqueous ethanol solution (50/50, v/v). The resulting solution was stored in a refrigerator at +4°C.

Liquid chromatographs Agilent 1260 Infinity with the diode-array detector, Spectra-Physics Spectra 100 UV detector, and fluorescence spectrometer Hitachi 850 with a flow cell as a detector have been used for the research and zinc bacitracin identification. Separation was performed on the columns ReproSil-Pur ODS 150 × 4 mm 5 µm, ReproSil ODS-AC 18 (5 µm) (250:4 mm), Equisil BDS-C18 (250 × 4.6 mm) in the mode of gradient elution of the mobile phase.

RESULTS AND DISCUSSION.

The issue to select an optimal composition of the mobile phase was one of the most important parts in the HPLC method of analysis development [12]. Several variants of systems with different concentrations and ratios were studied: from 45% to 55% 0.01 M ammonium acetate and from 45% to 100% methanol [13]. Further, the phase composition was changed in order to obtain the best separation with the minimal analysis time. Three-component systems were studied, such as acetonitrile-methanol (1:1 v/v) KH_2PO_4 aqueous solution (0.05 M, pH = 6.0) (49:51 v/v) and acetonitrile-methanol (1:1 v/v) KH_2PO_4 aqueous solution (0.05 M, pH = 6.0) (60:40 v/v). The latter system was selected as the optimal mobile phase for the chromatographic column Reprosil ODS—AC 18 (Pavli and Kmetec, 2001; 2004). BC components were completely separated according to the main peaks of BC-A, BC-B_1, BC-B_2 и BC-B_3, and BC-F. A, B_1, B_2, B_3.

The selection of conditions was carried out at a flow rate of 0.5 and 1.0 ml/minute. A chromatographic column with a reversed phase AC 18 (250:4 mm 5 µm) was used, the flow rate of 1.0 ml/minute was optimal at a thermostating temperature of 30°C. The analytical signal was recorded using a diode-matrix detector at the excitation wavelength of 245 nm (Fig. 1).

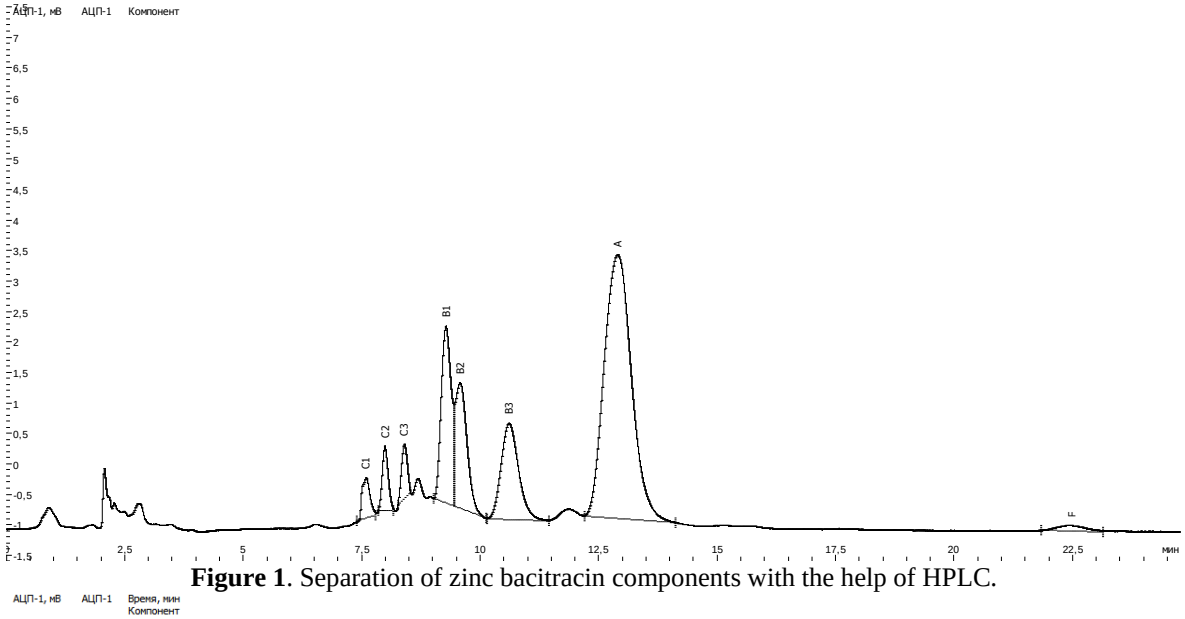

Figure 1. Separation of zinc bacitracin components with the help of HPLC.

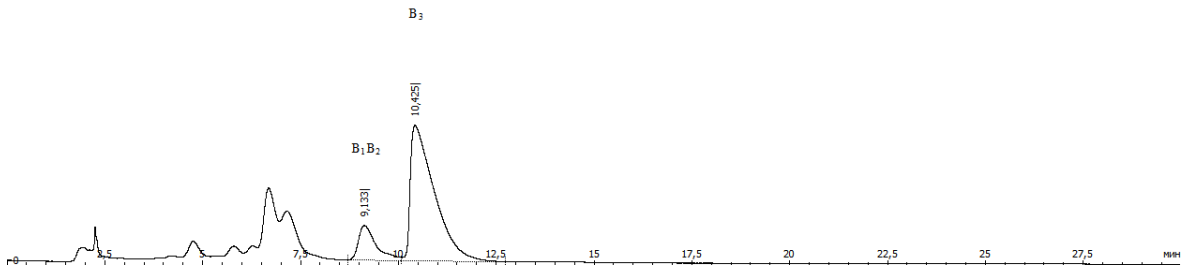

Figure 2. Chromatogram with the tested solution of 0.5% concentration without EDTA.

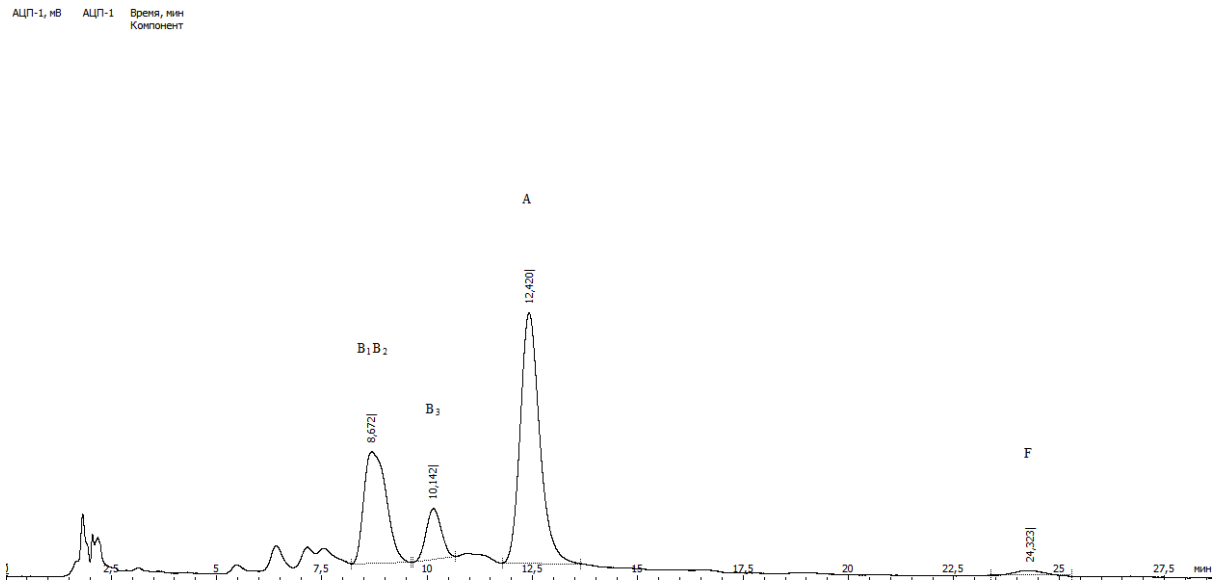

Figure 3. Chromatogram with EDTA added to the tested solution of 0.5% concentration.

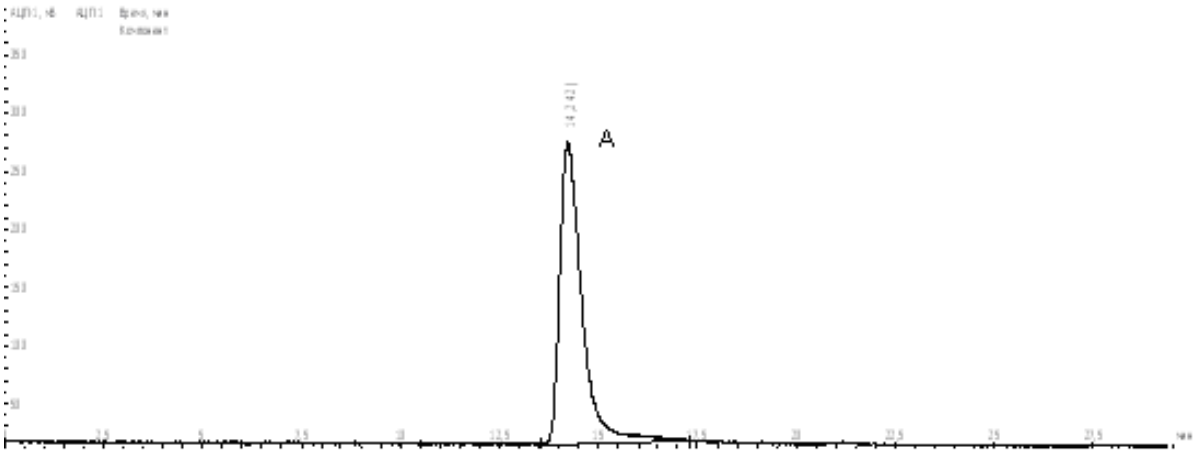

Figure 4. Separation of Zinc bacitracin components with the help of the fluorescence spectrometer Hitachi 850.

However, there was a problem of restoring the peak of bacitracin A at low concentrations. Bivalent metal cations, such as Fe^{2+} and Cu^{2+} that typically present in HPLC systems may interact with the bacitracin. This fact may explain its loss at low concentrations [14].

A 0.1 M solution of disodium EDTA salt was introduced in the aqueous part of the mobile phase in order to verify that the bacitracin low reduction was caused by the chelation with the metal ions. EDTA is commonly used as a strong metal-chelating agent for the bivalent metal cations present in the HPLC system (Alan et al., 2012).

The bacitracin maximum sensitivity was measured from 0.5% to 100% from the level of EDTA solution concentration [15].

Thus, EDTA addition to the mobile phase improved the interception to an acceptable level.

A chromatogram with the tested solution of 0.5% concentration without EDTA as a mobile phase modifier is presented in Figure 2.

These data indicate that the losses in the recovery process were caused by the interaction in the HPLC system.

A chromatogram with the tested solution of 0.5% concentration where the EDTA was added in the mobile phase is presented in Figure 3.

The peaks of bacitracins A, B_1, B_2, and B_3 were restored. The modified mobile phase was successful in bacitracin detecting at all concentrations necessary for the study.

In addition to hydroxyl and carbonyl groups, the zinc-bacitracin molecule also contains an amino group that can be derivatized to produce fluorescent compounds [16]. Primary amino groups derivatization in the side chain of the BC amino acids with orthophthaldehyde (OPA) in the presence of 2-mercaptoethanol was made to increase Zn-BC sensitivity. It gave an opportunity to enhance the fluorescence of the isoindoline products (Fig. 4).

CONCLUSION.

The optimum mobile phase has been chosen in the course of studies. Chromatography mode optimization for the Zn-BC indication in the standard samples has been carried out. Low detection of bacitracin A, B_1, B_2, and B_3 peaks is caused by the ability of this antibiotic to form complexes with metal ions in the HPLC system. A small amount of

EDTA addition prevented this phenomenon. The method of EDTA adding to the mobile phase increased the sensitivity of analysis in the ZnB detection. A method for ZnB identification in the standard samples by means of the phase-reversed HPLC and derivatization after a column with phthaldehyde before fluorescence detection was developed.

REFERENCES

[1] Baram G.I., Reichart D.V., Goldberg E.D., New features of high-performance liquid chromatography in pharmacopoeia analysis. Bull Exp Biol Med 2003, 135(1), 5–9.

[2] Capitan-Vallvey L.F., Titos A., High-performance liquid chromatography determination zinc bacitracin in animal feed by post-column derivatization and fluorescence detection. Chromatography. 2002, 3, 27–34.

[3] Galyautdinova G.G., Bosyakov V.I., Shangaraev N.G., Egorov V.I., Identification of feed antibiotic zinc bacitracin by means of HPLC. Sci Notes KSAVM. 2017, 232, 47-50.

[4] Galyautdinova G.G., Bosyakov V.I., Shangaraev N.G., Egorov V.I., Comparative analysis of the feed antibiotic zinc bacitracin. Veterinarian. 2017, 5, 5-9.

[5] Govaerts C., Orwa Cli J., Sequencing of bacitracin A and related minor components by liquid chromatography/electrospray ionization ion trap tandem mass spectrometry. Rapid Commun Mass Spectrom 2003, 17, 66–79.

[6] Hormazabal V., Yndestad M., Rapid assay for the determination of zinc bacitracin in feed by liquid chromatography—mass spectrometry. J Liq Chrom Rel Technol. 2000, 21(7), 3-8.

[7] Mansson M., Senstad C., Bacitracin antibiotics. United States Patent, Patent No.: 8410044 B2, 2013.

[8] Mascher D.G., Unger C.P., Mascher H.J., Determination of neomycin and bacitracin in human or rabbit serum by HPLC–MS/MS. Pharm Biomed. 2007, 43, 691–700.

[9] Ming L.J., Epperson J.D., Metal binding and structure–activity relationship of the metalloantibiotic peptide bacitracin. Inorg. Biochem. 2002, 91, 46–58.

[10] Oka H., Yamada M., Improvement of chemical analysis of antibiotics. Chromatography. 1989, 462, 15-22.

[11] Pavli V., Kmetec V., Optimization of HPLC method for stability testing of bacitracin. Pharm. Biomed. 2001, 24, 77-82.

[12] Pavli V., Kmetec V., Fast separation of bacitracin on monolithic silica columns. Pharm. Biomed. 2004, 36, 57-64.

[13] Potts A.R., Psurek T., Validation of quantitative HLPC method for bacitracin and zinc bacitracin using EDTA as a mobile-phase modifier. Pharm. Biomed. 2012, 70, 19-23.

[14] Sarri A.K., Megoulas N.C., Koupparis M.A., Development of a novel liquid chromatography–evaporative light scattering detection method for bacitracins and applications to quality control of pharmaceuticals. Anal Chim Acta. 2006, 2, 50–57.

[15] Sin D., Ho C., Wong C., Analysis of major components of residual bacitracin and colistin in food samples by liquid chromatography tandem mass spectrometry. Anal Chim. Acta. 2005, 5, 23-31.

[16] Sin D.M., Wong Y.C., Analytical methodologies for identifying a polypeptide antibiotic. Trends Anal Chem. 2003, 22(11), 799-809.