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DETERMINATION OF MOLECULAR PARAMETERS AND COLLIGATIVE PROPERTIES OF WATER SOLUTIONS OF ALBUMIN

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Summary: The comparative analysis of experimental data of electrophoretic division of proteinaceous preparations of blood by immudodiffusion methods is carried out to an agar and an electrophoresis on films from acetatecellulose, and also a valid assessment of results of researches.

Keywords: electrophoresis, immunoglobulin, albumine, monomers, highly effective liquid chromatography, validation.

Introduction

Proteinaceous preparations of blood of the person take leading positions in the market of medicines and are appointed by clinical physicians in large volumes therefore the requirements made to them, need some special attention.

Therefore an actual problem of pharmaceutical science is a development and improvement of methods of quality control and standardization of medicines. Blood of the person contains a difficult mixture of the proteins having functional differences, more than 130 of them are identified. The considerable part form them can be determined quantitatively by modern immunofermental and immunoturbidimetric methods. But the majority of them are inefficient or inaccessible because of rather high error and comparative high cost. Therefore standard shifts of proteinaceous composition of serum of blood can be determined by a much more available electrophoretic method, allowing to estimate an overall picture of a proteinaceous range and to receive the significant diagnostic information.

One of the most informative methods is the method of electrophoretic division which allows to define qualitative and quantitative composition of proteins.

The electrophoresis is a movement of the loaded particles under the influence of external electric field. Various proteins, possessing various molecular weight, under the influence of electric field move with a different speed. Albumine having the smallest molecular weight moves further from a place of drawing, then settles down α1-, α2-, β-and γ-globulin. Each of the main fractions can sometimes be divided into some subfractions. Speed of movement of particles depends on molecular weight, also on pH Wednesdays, properties of buffer solution and other factors. As the supporting environment carrier paper, a membrane from cellulose acetate, agar, starched or poliacryl gel or the combined environments can be applied. Now the large number of various electrophoretic methods which are based on properties of proteins and features of their interaction with the supporting environment applied at an electrophoresis has been developed.

Until recently the electrophoresis on paper was widely applied in many laboratories, however it has a number of disadvantages. The main of them is that results of fractionation of proteins can be received by this method only for 2-3 day of research. The complexity of preparation of gel, its high cost, and also difficulty of a quantitative assessment can possibly be refered to disadvantages of this method .

The electrophoresis on gel carriers yields significantly the best results, allowing to identify bigger number of proteinaceous fractions of serum (to 30), but it also has some disadvantages – the complexity of preparation of gel or high cost of ready gel plates are inherent in it. Use of membranes from acetate of cellulose allows to provide speed of the division of proteins and rather low cost of the analysis. As a whole application of acetate cellulose membranes allows to increase the clearness of fractionation and to reduce time required for the division, coloring and the analysis considerably.

The main indicator of quality of preparations of blood is preservation of high level of the maintenance of proteinaceous fractions in the course of production, their stability and a ratio of data of fractions in made pharmaceutical products.

Therefore the purpose of this research has become studying of methods of electrophoretic division of proteinaceous preparations of blood and a choice of most optimum of them for an assessment of quality of medicines.

As objects of research have been used the following: albumine solution for infusions of 10%, solution for intramuscular introduction of immunoglobulin of the person against tick-borne encephalitis made at Chelyabinsk regional station of blood transfusion.

Materials and methods. Definition of fractional structure and electrophoretic uniformity have been carried out by electrophoresis methods in agar gel of "Difko" company, films made of acetatecellulose.

Results and discussion. Determination of electrophoretic uniformity was carried out by an electrophoresis method on a film made of acetatecellulose on the UEF-01-ASTRA device, crimson Page was used as the dye. Vaseline oil and a mixture of ice acetic acid and acetone in the ratio 1:1 were served as clarifying solution, acid acetic was used as washing solution. The processing of the results of an

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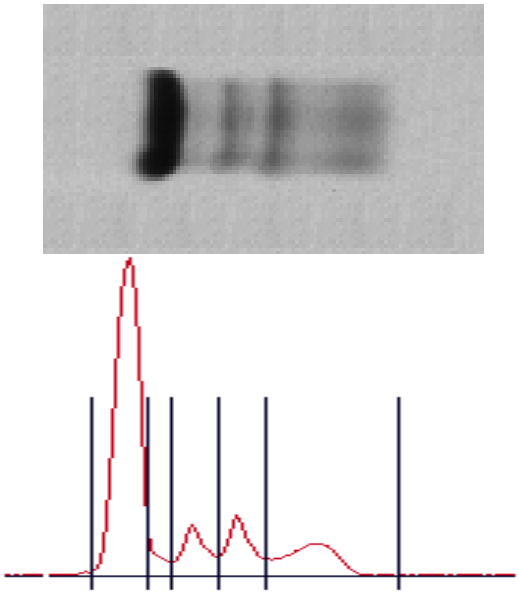
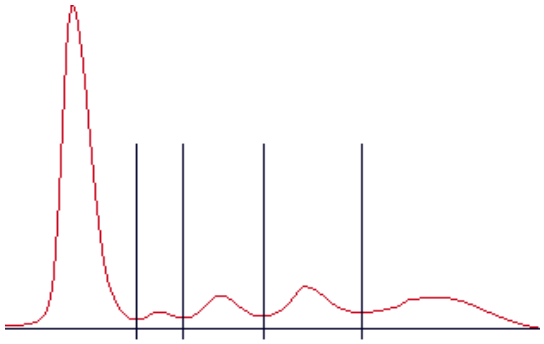
electrophoresis was made by means of a special program which together with the personal computer and the tablet scanner carries out densitometer functions. According to the results of researches of three series of preparations of immunoglobulin solution of a human being against tick-borne encephalitis on the electrophoregram only one intensive arch of a pretsipitation of Ig G and 4 additional arches is revealed. The contents of immunoglobulin G averaged 98,5 ± 0,3%, solution of albumine of 20% - 99,22 ± 0,3%. The specification of fractional structure was carried out by an immunoelectrophoresis method in agar gel. As reactants the following were used: Defco Laboratories firm "Difko's" agar, - buffer solution for an electrophoresis of proteins (Eco-service, KliniTest), anti-serums for an immunoelectrophoresis to proteins of plasma of blood of the person, allowed for the use by the Ministry of the Russian Federation. The bromphenol blue water-soluble was used as dye.

On the immunograms of three series of preparations: solution immunoglobulin of a human being against tick-borne encephalitis and solution of albumine of 20% only one intensive arch of a pretsipitation of Ig G and 3 additional arches (fig. 1 and 2) came to light.



Picture 1
Electrophoregram received on gel plates; it is painted by the bromphenol blue



Picture 2
Electrophoregram received on acetate cellulose membranes; it is painted crimson With

The comparison of results of electrophoregram proteins of serum of the blood, the membranes received with the use from acetate cellulose and agar gel, didn't reveal any essential distinctions. The use of membranes made of acetate of cellulose characterized by their low cost and availability for a short time allows to receive the accurate fractionation and a possibility of a quantitative assessment of proteinaceous fractions.

The quality of an electrophoresis and the received results depend on many factors: preparation of tests, the applied electrophoretic buffer, the quality of used acetate cellulose membranes, the quality of drawing tests on a membrane and amounts of the brought serum, used dye; a personnel skill level. That is why for a choice of an optimum method validation of used analytical techniques of an electrophoresis has been carried out.

During a valid assessment of analytical techniques the following indicators were analysed: convergence, intra laboratory accuracy, robastnost.

While determinating the convergence the criterion of an acceptability is the general relative standard deviation (RSD) which size has to be not lower or equal 1% . In experimental data when using electrophoresis on agar gel RSD was 1%, on a film made of acetatecellulose -0,9% that confirms the convergence of used techniques.

While determinating the intra laboratory reproducibility convergence of the experimental data obtained by different employees in one laboratory is satisfactory on condition of (RSD_{total}) 2,5%, in experimental data of (RSD_{total}) when using electrophoresis on agar gel - 2,3%, on a film made of acetatecellulose - 2%.

The received average meanings of two parallel series of the definitions which have been carried out by different employees in one laboratory, are statistically equivalent on condition of $\mathbf{t_{calculation} < t_{table}}$ (p=95%; f=m – 2), according to the data of the experiment when using electrophoresis on agar gel made tрасч.=0,995, tтабл=1,02, a film made of acetatecellulose tрасч.=0,987, tтабл.=0,997.

The analysis of robastnost showed the convergence of the results received at small changes in conditions of carrying out the analysis, criteria of an acceptability are satisfactory, under a condition, if RSD ≤ 1%. In experimental data of RSD_{total} when using

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electrophoresis on agar gel was 1%, on a film made of acetatecellulose 0,92%.

The valid assessment of methods proved that the electrophoresis on films made of acetatecellulose differs bigger reproducibility, a smaller error in represented results, and also less sensitive to external changes in experimental conditions. Besides considering the complexity of preparation of agar gel and high cost of ready gel plates, and also rather low cost of the analysis with the use of membranes from acetate of cellulose it is possible to draw a conclusion that the application of acetate cellulose membranes allows to increase fractionation clearness, provides the speed of division of protei; it is also possible to draw a conclusion that this technique is the most optimum for electrophoretic division, a quality and quantitative assessment of ready preparations of blood.</t_{table}>

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