INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Chemical Stability Study of the Benzylpenicillin Eye Drops compounding preparation by Thin Layer Chromatography

A. H. Valiev, O. A. Zdoryk, K. O. Khokhlova, V. A. Georgiyants.

Pharmaceutical chemistry department

National University of Pharmacy, Kharkiv, Ukraine.

ABSTRACT

The chemical stability of benzylpenicillin sodium in extemporaneously compounded eye drops and influence of stress testing by thin layer chromatography method were studied. It was established, that the benzylpenicillin sodium is affected by the acidic, basic, oxidative and UV light conditions. Three pilot batches of eye drops were kept at three environmental regimes over 14 days. It was shown, that benzylpenicillin eye drops are stable for 10 days under refrigeration (5±3 °C), allowing the convenience of infectious ophthalmic diseases treatment.

Keywords: Benzylpenicillin, compounding preparation, thin layer chromatography, stability.

INTRODUCTION

Despite the wide range of modern manufactured antibacterial drugs, compounding preparations of antibiotics are quite common prescribed. Compounding preparations, which contain penicillin, are widely used. According to the recommendations of the European Respiratory Society and the European Society of Clinical Microbiology and Infectious Diseases 2010, the use of penicillin is effective in treatment of variety of diseases, especially in combination therapy [1]. The most important conditions for the preparation of extemporaneous antibiotics solutions in pharmacy are sterility and stability. To achieve the sterility of extemporaneous solutions, the various methods of preparation are used. Among of them are addition of lyophilized powder for injection of industrial manufacture antibiotic and usage of sterile solvent [2]. Questions of stability study for compounding preparations remain of current interest for various frequently repeated prescriptions [3-5]. It is well known, that antibiotics are easily decomposed under the exposure of environmental factors, such as light, temperature, atmospheric oxygen. Such destructions may occur during compounding of dosage forms, its storage and usage by the patient.

During stability studies of the compounding preparations all attributes, that are susceptible to change throughout storage should be tested, especially those specifications that influence on quality, safety and/or efficacy. Above all the physical, chemical, biological, and microbiological attributes must be considered [6].

The aim of this paper is to study the chemical stability of benzylpenicillin sodium eye drops compounding preparation by thin layer chromatography (TLC) method.

MATERIALS AND METHODS

Benzylpenicillin eye drops compounding preparation were selected for the stability study. Composition: Benzylpenicillin Sodium 100000 IU, Solution of Magnesium sulphate 8 % - 10ml.

Preparation of benzylpenicillin eye drops: dissolve content of one benzylpenicillin sodium 1000000 IU vial in sterile 8% solution of magnesium sulphate, add the same diluting agent up to 100 ml of solution. The obtained solution was packed in vials of 10 ml and corked. Three pilot batches of eye drops were prepared in strict aseptic conditions, using a laminar

box (biological safety cabinet AC2-4E1 «Esco», Indonesia).

The chemical stability study of benzylpenicillin sodium eye drops compounding preparation and stress testing were carried out by TLC method. All solvents and reagents used in the study were analytical reagent grade, and all reagents used in the study were freshly prepared. TLC analysis was carried out according to requirements of the State Pharmacopoeia of Ukraine, which is harmonised with European one [7].

Thin-layer chromatographic Identification Test

Test solution. Solution of eye drops *(Solution A).*

Reference solution. Dissolve content of one benzylpenicillin sodium 1000000 IU vial in water R. The volume of solution is adjusted to 100 ml with the same solvent (*Solution B*).

Derivatization reagent. Iodine vapour.

Chromatographic conditions

Stationary phase. HPTLC plates silica gel of appropriate size (10*10 cm 10*5 cm), («Sorbfil», HPTLC-P-UV, Krasnodar, Russian Federation or analogues).

Mobile phase: toluene:ethyl acetate:glacial acetic acid (4:4:2).

Sample application. 4 mcL of each solution are applied as spots.

Development. Chromatographic chamber, saturated for 30 minutes with filter paper, developing distance 70-80 mm from lower edge of the plate.

Detection. Allow the plate to dry in air and expose it to iodine vapour until the spots appear.

Examination in white light.

Results. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution.

Stability study. Stability study of benzylpenicillin eye drops compounding preparation by TLC method was carried over 14 days: immediately after preparation, after 3, 7, 10 and 14 days. Three bottles of each batch were kept at three environmental regimes: 5±3 °C; 25 °C/60%±5RH (dark place); 25 °C/60%±5RH (day light).

Forced degradation study. One batch of benzylpenicillin eye drops was prepared for stress testing. It was stressed with acid, base, oxidative agent and UV light.

Preparation test solutions with stress factors:

Acidity degradation: Add 1 ml of 0.1 M Hydrochloric acid solution to 10 ml of Solution *A*, and stir (Solution 1).

Alkaline degradation: Add 1 ml of 0.1 M Sodium hydroxide solution to 10 ml of Solution *A*, and stir (Solution 2).

Oxidative degradation: Add 1 ml of 3% Hydrogen peroxide solution to 10 ml of Solution *A*, and stir (Solution 3).

UV degradation: 10 ml of Solution *A* put in closed UV chamber (365 nm) for one hour (Solution 4).

The solutions with stress factors were kept for one hour.

RESULTS AND DISCUSSION

As a result of TLC study of benzylpenicillin eye drops compounding preparation with magnesium sulphate *(Solution A)* in comparison with reference solution (Solution *B*) it was found, that the method is suitable for identification of benzylpenicillin sodium in the preparation, because ΔR_f = 0.02 (R_f *Solution A* = 0.61, and R_f *Solution B* = 0.59) (Fig.1).

For identification of possible degradation products and for establishing of the stability indicating power of the TLC stress testing was used. The results of forced degradation study of benzylpenicillin eye drops are given in the figure 2.

According to the results (figure 2), exposure of stress conditions (acid/base hydrolysis, oxidation, and UV light) lead to degradation of benzylpenicillin in eye drops. Changing of the spots shape of benzylpenicillin on the chromatogram (Fig. 2) indicates instability of the benzylpenicillin eye drops under the influence of UV-irradiation.

Stability study of the eye drops at the temperature of 30±2 °C/65%±5RH during 24 hours by TLC had shown that the preparation in the given conditions are not stable. Therefore, further stability study of the eye drops in the period of time was performed at three temperature conditions: 5±3 °C (in the refrigerator); 25±2 °C/60%±5 RH (day light); 25±2 °C/60%±5 RH (in dark place).

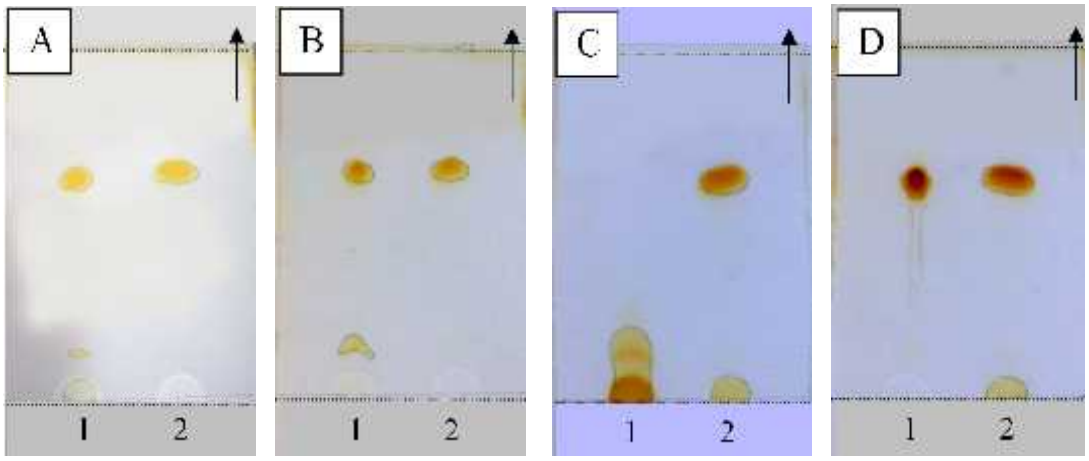
In figure 3 the results of stability study in time are shown. The preparation is stable for 10 days when stored at 5±3 °C, when protected from light. Solutions of benzylpenicillin sodium with magnesium sulphate stored at 25±2 °C/60%±5 RH (dark place and light) are instable.

CONCLUSION

According to the results of TLC chemical stability study of benzylpenicillin eye drops compounding preparation, the eye drops are stable for 10 days under refrigeration (5±3 °C). Under the exposure of external stress conditions, including acid/base hydrolysis, oxidation, and UV light solution of benzylpenicillin sodium with magnesium sulphate is not stable.

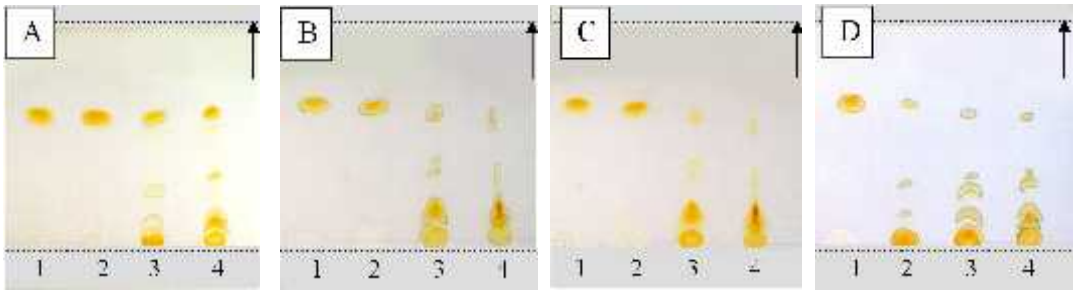
1 – Solution of benzylpenicillin sodium with magnesium sulfate (*Solution A*);
2 – Reference solution – solution of benzylpenicillin sodium (*Solution B*).

Figure 1
Chromatogram of benzylpenicillin eye drops compounding preparation



A – Acidity degradation, B – Alkaline degradation, C – Oxidative degradation, D – UV degradation.
1 – Solution of Benzylpenicillin eye drops compounding preparation after action of forced degradation agents 0.1 M Hydrochloric acid solution (A); 0.1 M Sodium hydroxide solution (B); 3% Hydrogen peroxide solution (C); UV light (365 nm) (D);
2 – Reference solution –solution of benzylpenicillin eye drops compounding preparation (*Solution A*) freshly prepared.

Figure 2
Forced degradation experiments of benzylpenicillin eye drops compounding preparation



A – 3 days, B – 7 days, C – 10 days , D – 14 days ;
1 –Solution of Benzylpenicillin eye drops with magnesium sulphate freshly prepared (*Solution A*);
2 – Solution of Benzylpenicillin eye drops with magnesium sulphate (storage conditions: t=5±3 °C);
3 – Solution of Benzylpenicillin eye drops with magnesium sulphate (storage conditions: t=25±2 °C /60%±5 RH, day light);
4 – Solution of Benzylpenicillin eye drops with magnesium sulphate (storage conditions: t=25±2 °C/60%±5 RH, dark place).

Fig. 3
Stability study of benzylpenicillin eye drops compounding preparation pilot batches during 14 days

REFERENCES

1. Sinopalnikov AI, Romanovskikh AG, The Management of Lower Respiratory Tract Infections in Adult Patients, Clinical Microbiology and Antimicrobial Chemotherapy. 2012; 14(1): 4-16.
2. A guide for compounding practitioner, The United States Pharmacopeial Convention, Rockville, 2012, 317 p.
3. Zdoryk OA, World experience of the expiration date determination of compounding preparations, Pharmaceutical journal. 2013; 6: 42-47.
4. McDougall David AJ, McWhinney Brett C, Stability of buffered benzylpenicillin solutions for outpatient parenteral antimicrobial therapy, Journal of Pharmacy Practice and Research. 2014; 44(1): 26-28.
5. Zdoryk OA, Khokhlova KO, Georgiyants VA, Vyshnevska LI, Investigation of Physical and Chemical Stability of Ointment with Herbals, IJPC. 2014; 18(3): 248-252.
6. CPMP/ICH/2736/99 (ICH Topic Q1A (R2)) Note for Guidance on Stability Testing: Stability Testing of New Drug Substances and Products, 2003.
7. Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. State Pharmacopoeia of Ukraine. 1st ed. Kharkov City, Ukraine; 2001. 556 p.