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TCH036

Development and validation of a method to study the mixture daptomycin/heparin in ringer lactato by High Performance Liquid Chromatography (HPLC)

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Background

In patients with infection related to catheter (IRC) is needed to consider several aspects that will lead both the managing and treatment of them. When the retention of the catheter is advisable it is necessary to apply technologies of sealed antibiotic. With that aim, the mixture daptomycin/ heparin prepared in ringer lactate solution has been proposed to be used in a long term stability study.

Purpose

This research has been focused on the stability study of the mixture daptomycin/heparin sodium prepared in ringer lactate solution since no information upon its chemical and physical compatibility has been found in the consulted bibliography. A validated indicating high performance liquid chromatography method (HPLC) has been developed following the International Conference on Harmonization guidelines (ICHs) for this purpose.

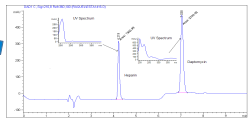
Methods

A validated HPLC stability-indicating method for determining the mixture daptomycin/heparin prepared in ringer lactato has been developed. The ICH guidelines (ICH, Q2(R1) 2005 and ICH, Q1A(R2) 2003) has been followed.

Chromatographic system (1100 Agilent liquid chromatograph equipped)

Chromatographic analysis were carried in a 250 mm x 4.6 mm i.d., 5µm particular size C18 ODS analytical column, using a mobile phase containing water with 1% trifluoroacetic/acetonitrile. The application of a gradient was necessary to achieve drugs separations. The temperature of the column was set at 25 ºC. 5 µL of sample were injected into the chromatograph.

The chromatograms were registered (Diode Array Detector) at 210.8 ± 50 nm, using 360 ± 20 nm as reference wavelength. UV spectra were taken every 5 seconds.



Chromatogram of **daptomycin** and **heparin** in ringer lactate (500mg/L of daptomycin and 10mg/L of heparin) at the instrumental HPLC conditions selected.

Analytical method validation

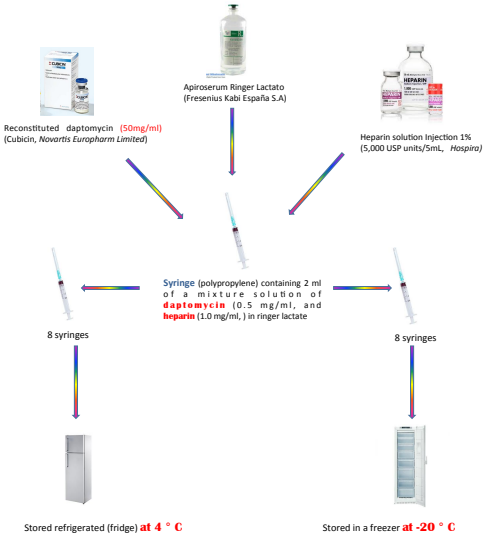
To perform the validation of the method the following parameters were evaluated:

- Linearity
- Limits of Detection and Quantification
- Precision
- Accuracy
- Specificity. The stress conditions studied were: heat (temperature), UV light, acid, base and oxidant media
- Robustness
- System suitability

Once it was proved the suitability of the method as stability indicating, it was used to study the chemical stability of the mixture daptomycin/heparin in ringer lactato and stored in syringe at different storage conditions.

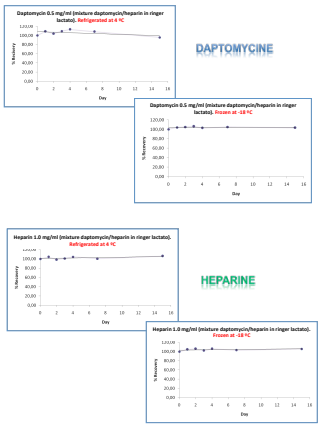
Stability study of the mixture Daptomycin/Heparin

Preparation of the samples for storage and HPLC analysis



Appropriate volumes of this samples were injected into the chromatograph system under the instrumental conditions previously established. The check times for analysis were 0 , 1 , 2 , 3 , 4 , 7 and 15 days after their preparation.

Results and Discussion



The figures show the evolution of the daptomycin and heparin concentrations for the two storage conditions studied. In this chemical stability study, the concentration of the drug was expressed as the percentage of the remaining drug concentration at different times, under the different conservation conditions studied. In all cases the measured concentration was the average of three replicates. The stability was assumed if the loss was less than 10% of the initial concentration. The concentrations obtained by the analysis of the mixture immediately after their preparation were considered as the initial concentrations.

It could state that daptomycin and heparin concentrations did not change significantly during the fifteen days studied. All the obtained results were within ± 10 % of the label claim of the initial concentration.

CONCLUSION

THE PROPOSED HPLC METHOD WAS PRECISE, SPECIFIC, ACCURATE AND STABILITY INDICATING. THE MIXTURE DAPTOMYCIN/HEPARINE PREPARED IN RINGER LACTATO COULD BE DETERMINED SUCCESSFULLY , ALSO IN THE PRESENCE OF DEGRADATION PRODUCTS. THE ICH GUIDELINES WERE FOLLOWED THROUGHOUT THE STUDY FOR THE METHOD VALIDATION AND STRESS TESTING.

ONCE THE METHOD WAS VALIDATED AS STABILITY INDICATING, IT WAS USED TO STUDY THE STABILITY OF THE MIXTURES IN PRELOADED SYRINGES AT DIFFERENT STORAGES CONDITIONS. REGARDING THIS STUDY, THE RESULTS SHOWED THAT THE DRUG STORED IN PRELOADED SYRINGES FOR 15 DAYS, WERE CHEMICALLY AND PHYSICALLY STABLE IN THE DIFFERENT CONSERVATION CONDITIONS STUDIED. THUS, IT COULD CONCLUDED THAT THE MIXTURE DAPTOMYCIN/HEPARIN IN RINGER LACTATO IN PRELOADED SYRINGES WAS PHARMACEUTICALLY STABLE AND SUITABLE FOR USE AT LEAST 15 DAYS AFTER THEIR PREPARATION.

Acknowledgment

Financial support was provided by the Project PI 101 / 00201 (Instituto de Salud Carlos III, Ministerio de Ciencia e Innovacion, Spain)