Validation of an HPLC method to quantify D-penicillamine

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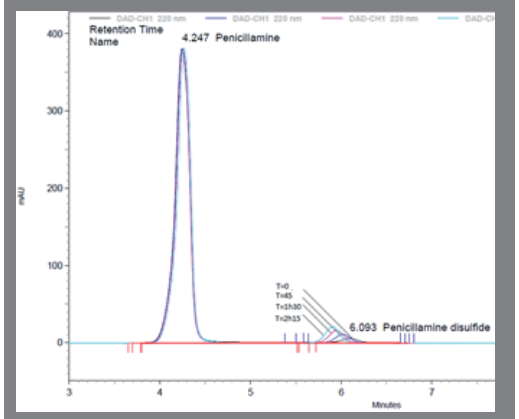
Introduction

Nowadays, generic drugs have become a real alternative. Indeed, they have the same effects as reference drugs called brand drugs, but at a lower cost. In this favorable context, the pharmaceutical laboratory MAP France develops and analyzes new drugs. We were focused on Trolovol whose active ingredient is D-penicillamine. This drug is specifically prescribed for the treatment of Wilson's disease and rheumatoid arthritis. Thus the aim of this experiment was to validate a new method of analysis by HPLC, following a protocol which includes a statistical test.

Experimental procedures

Analyses were performed on a LaChrom Elite HPLC system (High performance Liquid Chromatography) coupled with a DAD detector from Hatachi technology (UV detection was carried out at 220 nm). The column used was a C18, size 150x3.9 mm, with a flow rate of 0.8 ml/min. 20µL of solution were injected, and the mixture was separated using a mobile phase made up of 100% potassium dihydrogenophosphate (6.8g/L) and hexansulfonic acid (0.206g/L) buffer whose pH was adjusted to 3.5. So under these conditions the run ended in 15 minutes.
The theoretical concentration of D-penicillamine required for the assay was 3 mg/ml.

Stability of D-penicillamine (superposition of multiple chromatograms at T=0 min (black), T=45min (blue), T=1h30 (pink), T=2h15min (skyblue).)



Results and discussion

ESeveral parameters such as specificity, linearity, repeatability, reproducibility, accuracy and stability were studied in order to validate the analytical method.
As shown in the Figure hereafter, D-penicillamine and the main impurity D-penicillamine disulfide are well separated. This chromatogram also reveals the instability of D-penicillamine due to the fact that it is degraded into an impurity over time. This characteristic is important to note, it means that D-penicillamine cannot be preserved in solution.
Firstly, a solution of the active ingredient and one of impurity were prepared to check the specificity.
Secondly, after comparison of the slopes and the Y-intercepts of the linear regressions of the active ingredient with and without all excipients, it concluded that the experiment could only be carried out in water. It means the response of D-penicillamine in the chromatographic system is linear within the range [80%-120%] of the concentration used in the assay and there is no matrix effect.
Thirdly, repeatability was tested with 10 injections on the same day, and reproducibility was conducted for 3 consecutive days. Accuracy is deduced from statistical calculations using the previous test results for repeatability and the confidence interval can therefore be estimated.

Conclusion

Several coefficients of variation (reproducibility and repeatability) were under 2%, so the method seemed reproducible, repeatable and exact with a confidence interval of 0.19%. All parameters were successfully tested and this analytical method was validated. Thus, in the future, it will be necessary to carry out a forced degradation of the drug (PH, temperature, with H_2O_2) in order to determine the ideal conditions of storage and dissolution.

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