Developing a High Performance Liquid Chromatography method to quantify Vancomycin.

Fanny DAYOT

Introduction

The subject deals with a punctual study which was requested by the AFSSAPS which is a French agency responsible for monitoring health products.
The aim was to realize the assay of vancomycin, especially its degradation products, in HPLC coupled with UV-Visible detector.
Vancomycin is an antibiotic that has an effect just on bacteria Gram positive. But it seems that generics of vancomycin (copy) are less efficient than princeps (original).
Indeed, it is likely to arise from vancomycin degradation products which would prevent the antibiotic to fix on its target. Only the first point of the objective is treated by this article, adjusting a method in order to quantify the vancomycin.

Experimental methods

Liquid chromatography consists in separating compounds of a mixture.
As a rule, regulating chromatographic conditions permit compounds to be eluted at different retention times.
The chromatographic characteristics are summarized hereafter:

Parameters	Conditions
Column	Symmetry C8 100A 5.0µm 4,6mm*250mm WATERS
Mobile Phases	**Phase A** : 91% Buffer/ 5% ACN/ 4% MetOH **Phase B** : 84% Buffer/ 8% ACN/ 8% MetOH
Oven Temperature	22°C
Volume of injection	50 µl
Flow rate	1 ml/min
Detection	UV-Visible

Moreover, a liquid/liquid extraction was used with three solvents of extraction: Isopropanol, Acetonitrile, and Methanol.

Results and discussions

An internal calibration was realized for more sensitivity, to quantify the vancomycin.
A good separation and resolution were obtained between the internal standard, caffeine, and vancomycin. (Figure 1)

Furthermore, after a lot of tests, a range of calibration was prepared and the result was a linear and repeatable straight line. However, the reproducibility of this method was not demonstrated. (Figure 2)

Finally, a gradient of mobile phases was used to reduce the run, so the time of analysis was acceptable.

Figure 1: Chromatogram of a higher point of calibration (200 mg/l)

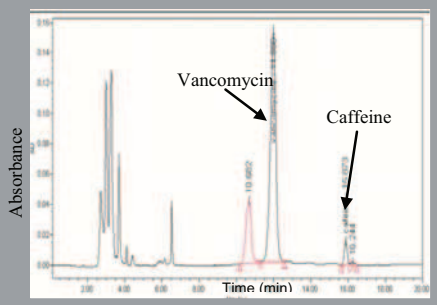
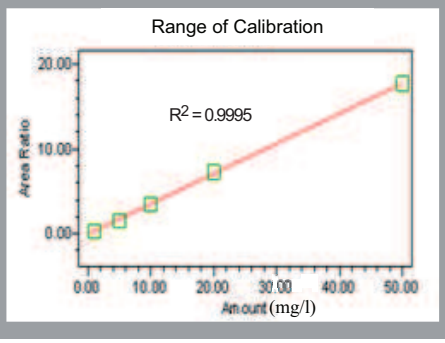


Figure 2: Range of vancomycin calibration (from 0 mg/l to 50 mg/l)



Conclusion

Controls are close to expected concentrations. After having estimated the reproducibility, the purpose will be to quantify and compare the concentration of degradation products of vancomycin in generic and princep antibiotics, using the previous method of dosage.

Laboratoire de Toxicologie-Pharmacologie
CHU de Dijon