Validation of an analytical method for the titration of anti retroviral drugs

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Introduction

By 2016, every medical biology laboratory must be accredited with the ISO 15189 norm. In 2013, hospitals will have to show that they have already begun the process of accreditation. This is an obligation, all laboratories which do not have the ISO 15189 accreditation by the deadline will have to close. Consequently, the pharmacology laboratory of Edouard Herriot Hospital has to validate all the anti-retroviral molecules which are titrated according to the ISO 15189.

Experimental Conditions

The four molecules: Lopinavir (LPV), Ritonavir (RTV), Atazanavir (ATV), and Efavirenz (EFV) were separated by Liquid Chromatography coupled with Mass Spectrometry (LC-MS) in reversed phase chromatography. The detection system was a UV-detector coupled with a mass detector. The drugs were separated by increasing the composition in acetonitrile of the mobile phase thus corresponding to a solvent gradient. The validation consists in analyzing the blood of patients who are positive HIV, with the LC-MS and also with the instrument which was used before in order to compare the results. The quality control was also analyzed in order to verify the reliability of the analysis.

Chromatography Parameters :

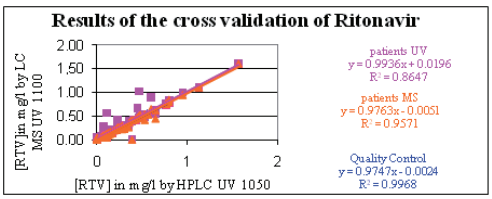
- use of different mobile phases
- mode: constant flow
- heated column
- use of an internal standard

Due to the confidentiality of the method, LC-MS parameters are not given.

Results and discussion

The comparison of HPLC-UV and LC-MS ensures that the UV value and the average of the UV and mass detector value give the same results for all patients. Graph represents the comparison of UV (HPLC)-UV (LCMS), and UV (HPLC)-MS (LCMS) results for patients and quality control. It is important to compare the cross validation of quality control and patient because quality control are in a lyophylisate form. We observe from the graph that for low concentrations of Ritonavir of the patients, the mass detector is more sensitive than the UV detector. The inconvenience of the mass detector is that it can fluctuate with time. So it is necessary to analyze quality control at the beginning and at the end of the experiment in order to verify the stability of the mass detector.
We observe that the two detectors give results which are quite similar even if the correlation factor is about 0.86 with UV, 0.95 with MS and 0.99 for Quality Control.

The correlation factor cannot be a parameter for a cross validation; it is only an indicator in order to see whether the results are similar or not. The results of the cross validation will be analyzed by a specific software.



Graph 1: Comparison of the concentration of Lopinavir between UV and MS detector.