ADAPTATION OF THE ASSAY OF HYDROXYCHLOROQUINE BY LC-MS

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Introduction

Hydroxychloroquine is a drug which is prescribed to patients who suffer from rheumatoid arthritis and lupus. It is also used to treat malaria and must be taken daily. If not, the symptoms will reappear and an hospitalization will be necessary to cure the patients. It is important to assay hydroxychloroquine into the patients' blood to be sure they take the medicine as they are supposed to do.
The aim of this article is to outline a method to identify and quantify the hydroxychloroquine. Firstly in serum and in whole blood then despite the difficulty to identify and quantify the drug in whole blood due to the matrix effects.

Experimental procedures

100µL of the internal standard were added to 400µL of serum and after proteins were precipitated by 500µL of acetonitrile. After two imperative centrifugations to separate the solid to the liquid phase, sample preparation was performed by solid phase extraction on Turboflow® column. This column allows to eliminate the molecules of no interest and to retain toxins or drugs such as hydroxychloroquine. The analysis was carried out by a Liquid Chromatography coupled with mass spectrometry. The sample molecules were separated by a C18 Hypersil Gold PFP (100 x 3mm) column, ionized by an electrospray source and analyzed by a linear ion trap. Identification of molecules is possible thanks to their retention time and mass spectrum. Also, the quantification can be found by the ratio between the peak intensity of the hydroxychloroquine and the peak intensity of the internal standard.

Results and discussion

In a first hand we tested Chloroquine, Prazepam-D5 and Hydroxychloroquine-D4 as internal standards. We calculated the slot and the response factor of calibrations ranges at 0.2/0.5 and 1mg/L in hydroxychloroquine with 100µL of an internal standard. It appeared that Chloroquine was not reducing the matrix effects and a lack of intensity of the drug, with Hydroxychloroquine-D4, occurred at point 0.2mg/L. Prazepam-D5 had the best stability, determination coefficient and a response factor equal to 1.

Secondly, to identify and quantify the Hydroxychloroquine in the whole blood, another sample preparation was required. To be able to analyze the sample, the liquid injected into the LC-MS must be colorless. We tried several quantities of acetonitrile in order to obtain a colorless solution. Due to the huge amount of proteins in the whole blood, a volume of 1.5mL in acetonitrile was necessary.

Conclusion

Identification and quantification of hydroxychloroquine are important and possible with a LC-MS instrumental in serum. However, in whole blood matrix another sample preparation is required. Prazepam-D5 is the best internal standard using the method described previously.

Hydoxychloroquine's Intensity/Prazepam-D5's Intensity = f([Hydroxychloroquine])

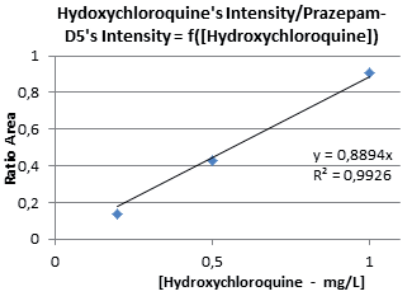

Figure 1: Calibration range in Hydroxychloroquine with Prazepam-D5

Volume of ACN (µL)	500	1000	1500
Color of the solution	red	yellow	colorless

Figure 2: Volume of Acetonitrile in whole blood sample

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