Validation of A Quantification Method for Drug X in Rat Plasma by LC-MS/MS

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Introduction

Before obtaining permission to market, new therapeutic molecules have to be tested. Two successive test phases exist: pre-clinical assays and clinical assays. Pre clinical tests are performed on Laboratory animals, in order to study the maximum tolerance, toxicity, metabolism and the pharmacokinetic of each new molecule.
During these studies, it is essential to use well defined and fully validated quantification methods to obtain reliable results that can be satisfactorily interpreted. In this case study , therapeutic molecule X was administered to rats and, some plasma samples were collected at different dates and hours. Development of an analytical method was necessary to quantify drug X. Nevertheless, it was essential that this method was validated before being used. Method validation is a key stage in bio analysis; it demonstrates the quality of the analysis. To this end, the analytical method has to be evaluated according to several criteria: repeatability, linearity, reproducibility, accuracy, recovery, limits of quantification, matrix effect and stability assays.

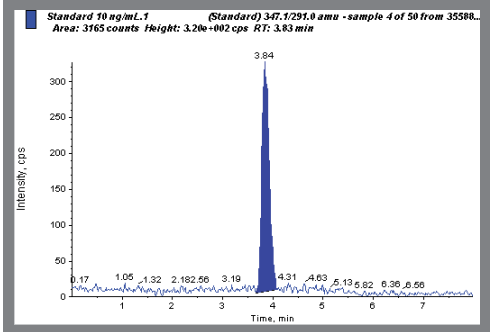
Experimental Conditions

The analyses were performed using high-performance liquid chromatography-tandem mass spectrometry.
The chromatographic system was an Agilent Series 1100 equipped with binary pumps. Separation was obtained with a Zorbax XDB Phenyl (Agilent: 3.5µm; 3.0 x 150mm) whose stationary phase was specific for drug X. The mobile phase used contained 60% acetonitrile and 40% water, at a flow of 200 µL/minute. The total analysis time was 13 minutes. Detection was carried out by an Applied Biosystems API 2000 mass spectrometer equipped with an electrospray source (positive ionisation) and a triple-quadrupole analyser.
The analysis mode for MS/MS was based on the multiple reactions monitoring method (MRM). The drug X extraction method from rat plasma was performed with Solid Phase Extraction SPE. Electrical interactions between the ionized form of drug X and stationary phase contained in extraction cartridges allowed drug X to be held back. Finally, drug X had to be dissolved in 2% formic acid water before being injected into the HPLC-MS/MS system. In order to quantify drug X in plasma samples, it was necessary to build a calibration range with calibration standards and quality control samples.

Results and discussion

All the validation tests demonstrated that the quantification method was sensitive from 10 ng/mL to 10 000 ng/mL. Figure 1 shows a Low Limit of Quantification chromatogram at 3.84 min.
There was also a linear relation between detector response and drug X concentration in rat plasma. Moreover, the method was specific and selective towards drug X and the internal standard Y in plasma.
An average recovery (approximately 60% for drug X and also for Y) was obtained as a result of the extraction, but it was demonstrated that this result is very constant. Intra-day and inter-day accuracy and precision tests were valid, consequently the method was repeatable and reliable. Matrix and dilution effects were not significant in this study. In addition, drug X was stable in plasma and in a solution under different storage conditions.

Figure 1: Chromatogram of Drug X at Low Limit Of Quantification LLOQ : retention time = 3,84 min.



Conclusion

This quantification method was validated and can be used in daily analysis for drug X in rat plasma. High performance liquid chromatography-tandem mass spectrometry guarantees very specific, selective and sensitive analysis for this drug X in a reduced analysis time.

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