VALIDATION OF A HILIC METHOD FOR THE DETERMINATION OF CIDOFOVIR IN PLASMA

F. Lecomte[1], C. Jasselette[1], C. Hubert[1], S. Demarche[1], C. De Bleye[1], A. Ceccato[2], E. Rozet[1], <u>Ph. Hubert[1]</u>

[1] Laboratory of Analytical Chemistry, CIRM, Institute of Pharmacy, University of Liège, Belgium
[2] Odyssea Pharma, Belgium

Cidofovir (CDV) is an acyclic cytidine monophosphate analog that as a broad antiviral spectrum including herpes viruses, adenoviruses, poxviruses, papillomavirus, and hepadnaviruses. Topical administration of cidofovir has been shown to be effective in the treatment of cutaneous infections of different viruses in animal models [1-2]. CDV is currently undergoing evaluation in clinical trials as a topic agent for treatment of papillomavirus infections [1,3]. An important adverse effect associeted with intravenous cidofovir is renal tubular damage. The main goal of this method was the CDV determination in human plasma after topical treatment so as to study the distribution in human body. Cidofovir is a polar molecule that has three ionizable functions. CDV present a zwitterionic character in aqueous media and therefore, it was an excellent candidate for determination by hydrophilic interaction chromatography (HILIC). This mode is an interesting alternative to reverse-phase liquid chromatography for the analysis of ionizable compounds [4]. The analytical conditions were optimized by means of designs of experiments.The bare silica column selected was a Grace Alltima HP HILIC. The isocratic separation was performed at a temperature of 25°C using a mobile phase consisting in a mixture of acetonitrile – 20 mM ammonium hydrogen carbonate buffer at pH 7 (72/28, v/v). Validation should ensure that the analytical procedure is fit for its purpose. In this application the aim of the developed method is to quantify CDV in plasma. A total error approach was used to demonstrate the fitness of the method using tolerance interval methodology and accuracy profile as decision tool. The tolerance interval used is a "β-expectation tolerance interval" defining an interval in which it is expected that each future result will fall with a defined probability β. It is therefore a predictive tool [5]. The concept of accuracy profile was also used to select the most appropriate regression model for calibration, to determine the lower limit of quantitation (LLOQ) and the range over which the method can be considered as valid. This newly developed method was then fully validated according to FDA requirements [6] by means of a Total Error approach that guaranteed that each future result will fall within acceptance limits of ±30% with a minimum probability β settled at 95% over a concentration range of 100 to 1020 ng/ml. Nonetheless, the routine application of the cidofovir assay in a pre-clinical trial demonstrated that the prediction made during the pre-study validation was consistent. Actually the minimum probability to observe QC samples within the ±30% acceptance limits was successfully of 95%.

References

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