Optimising and validating a method for the HPLC assay of an active molecule in order to achieve its forced degradation

Camille GARNAUD

Introduction

Currently, the prescription and consumption of generic drugs are booming. Indeed, world's population is continuously ageing, it causes an increase in drugs requirement which is difficult to finance. Generic drugs appear as the best solution, providing the same quality care as the original drug called princeps, with the same quality and quantity of active molecules, with a 30 to 40% lower price

The sale of a generic drug is possible only after obtaining an Authorization of Launch on the Market (ALM). In order to acquire the ALM for a corticoid, which the active molecule is the dexamethasone sodium phosphate, an assay method of this compound should be developed and validated following a validation protocol. After this, a forced degradation of this active molecule was done to establish manufacture conditions of the final product.

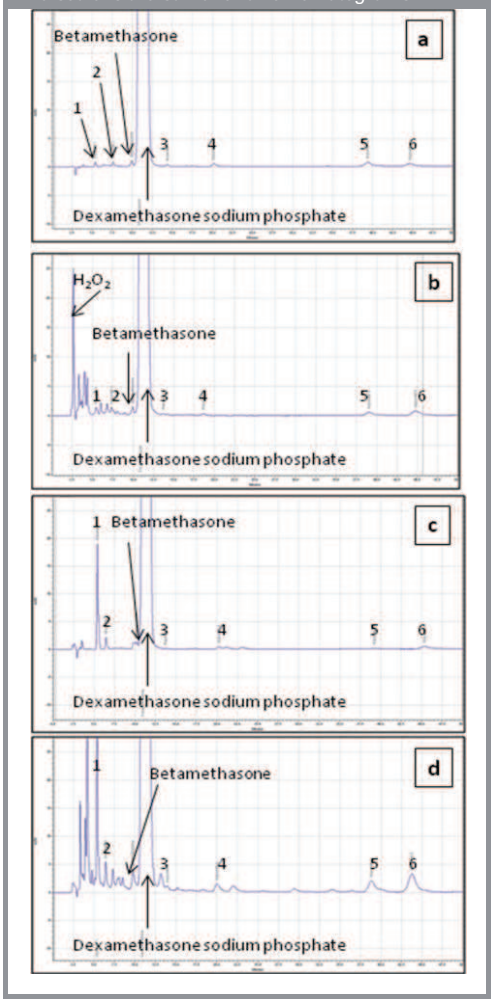
Experimental methods

The column used was an ACE 5 C18 endcapped, 250mm x 4.6mm x 5µm on an HPLC instrument VWR Hitachi, Elite LaChrom. The separation was obtained with a mobile phase containing 75% of ammonium formate buffer (1.32g/L, pH adjusted to 3.6 with formic acid), and 25% of acetonitrile at a 1mL/min flow rate, 20µL were injected and the chromatographic run was 50 minutes. The detection wavelength was 254nm. This method was validated according to the laboratory protocol, which included define linearity, repeatability, reproducibility, accuracy and stability.

Results and discussion

The dexamethasone sodium phosphate had seven main impurities (fig 1a). Chromatograms demonstrated that it was unstable at basic pH (fig 1c), it was sensitive to oxidation (fig 1b) and high temperature (fig 1d). Indeed, many other impurities appeared, and the active molecule was degraded. This implies that the manufacture of this generic drug has to be conducted in an inert environment and the finished product must be acidified and sterilized by filtration.

Figure 1 : Chromatograms of dexamethasone sodium phosphate in different conditions : a) reference, b) with H_2O_2 after 48h, c) pH adjusted to 11.5, d) after 25 minutes at 130°C.
The scale is the same for all chromatograms.



Conclusion

In conclusion, this method could be routinely used to assay dexamethasone sodium phosphate. A study was set up in the laboratory to establish a matrix within which the active molecule will be more stable and resistant to extreme conditions than the current one.

Laboratoire MAP France
14 rue Jacques Monod
69007 Lyon, FRANCE