Developing and validating an HPLC method to quantify an IPA (Ingredient Pharmaceutical Active).

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Introduction

MAP France is a pharmaceutical laboratory which performs assays in the field of generic drugs. Their work steps in after the drug patent expiration, and their goal is to develop new generic drugs with the same molecule. This project is focused on an IPA, often used in hospitals, particularly for ventricular failures or pulmonary oedema. The aim of this work was to develop an analytical method with the best operating conditions and to validate it.

Experimental conditions

The chemical analyses were performed with the High Performance Liquid Chromatography, coupled with a UV detector;

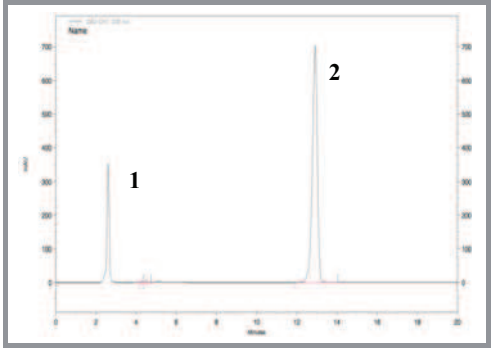
Instrument:	Elite Lachrom Hitachi
Column:	Symta C18 (250x4,6 mm) with particles of 5 µm
Flow rate:	1 ml/min
Injection volume:	20 µL
Solvent:	Methanol/Water (40/60)
Detection:	UV at 220 nm

The aim was to validate this method as a result of various statistic tests. Eight parameters were tested, such as ; linearity, accuracy, repeatability, reproducibility or stability.
In order to optimise time and analyses, an experiment plan was prepared. For all the analyses, the IPA was diluted to 0.5 mg/mL in water, except for the linearity. This parameter required a concentration range between 0.4 and 0.6 mg/mL.

Results and discussion

The detection of this molecule was easily performed with the established HPLC method. As shown in Figure 1, an excellent detection of the IPA and its various excipients was obtained, without any interference.
During the validation, many problems were noted for the sample preparations. Therefore, all analyses were realized at a lower concentration than the original drug. These dilutions led to eventual errors in stability analyses. However, all parameters were evaluated.
First, a solution of all the excipients with the IPA were analysed in order to check the specificity. Then, a comparison between two linearities (in water and in all excipients) allowed us to decide whether the other analyses should be performed in water only.
Furthermore, three different parameters were studied (accuracy, exactitude and sensitivity) that resulted directly from previous assays, and depended on the statistics. Studies were conducted for 3 consecutive days and after a repetition on the same day. This allowed us to check the repeatability and the reproducibility through the coefficient of variation (CV). Finally, a solution was prepared, analyzed and then left at 25°C for 24 hours. A second analysis allowed us to verify the stability so that routine tests were carried out without deterioration.

Figure 1 : Chromatogram obtained after the development :
1 = Excipients (mannitol and sodium acetate)
2 = IPA at 1 mg/ml



Conclusion

The developed analytical method was successfully validated. Indeed, all the validation criteria were approved for this molecule. The HPLC method allowed analyses to be repeatable and reproducible over several days (CV<2%). The method was also accurate and correct; it could recover the amount of IPA which was initially dissolved (100%).
What is more, successive analyses during 24 hours showed a great stability of the product at 0.5 mg/mL.
Finally, the method was linear with and without any matrix. This parameter was very considerable, because the future analyses will be simplified, since they will be carried out in water, without adding any excipients. This means that this method could be used again and again during the stability assays involved in all developments of new drugs.

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