QUANTITATIVE PERFORMANCES OF UHPSFC AND UHPLC : A COMPARATIVE STUDY

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Nowadays, the interest of supercritical fluid chromatography (SFC), such as improved chromatographic performances, greener technique and high throughput, are worldwide approved. Even if SFC was hidden in the shadow of gas chromatography (GC) and liquid chromatography (LC) for a long time, the recent increase of publications number reveals its resurgence of interest and relevance. However, scientists did not truly focus their work on quantitative performances evaluation but more on fundamental studies and chiral applications [1-2].

The concept of Quality by Design (QbD) is now well established in the pharmaceutical development and defined by ICH Q8 R2 as "a systematic approach to development that begins with pre-defined objectives and emphasises product and process understanding based on sound science and quality risk management". In this concept, the Design Space (DS) was introduced as a key component of analytical method development. In chromatographic words, the DS could be defined as the space of chromatographic conditions that will ensure the quality of the separation, thus the robustness is guaranteed inside the DS limits. Then, method validation could be performed directly after robust method optimisation by means of Design Space strategy, allowing speeding up the analytical life cycle.

In order to evaluate the interest of SFC as a quantitative technique, antibiotics were selected as model compounds because of their importance in the pharmaceutical field and their various structures and chemical properties. Firstly, a screening experimental design was performed in order to select the nature of stationary and mobile phases, BEH 2-ethylpyridine as stationary phase and methanol as modifier were selected. Then, a three factors rotatable central composite design was planed to optimise the separation. These three parameters impacting on the gradient profile allow tuning the selectivity of the method. The retention times at the beginning, the apex and the end of each peaks were modelled and the Design Space was computed using $S > 0$ (separation between critical pair of peaks) as critical quality attribute. Then the optimal chromatographic condition was experimentally tested showing the baseline separation between all peaks. This optimal condition was validated according to the total error approach [3]. In the same way, an UHPLC method was previously developed using DS strategy [4]. The UHPSFC and UHPLC methods were validated for the same pharmaceutical formulation: amoxicilline capsules. The selectivity was evaluated prior to the validation showing no matrix effect. On the other hand, the retention of amoxicilline is quite low in UHPLC (k_{app}= 1.5) while this compound is well retained in SFC (k_{app}= 14.8). UHPSFC is a really interesting technique for compounds showing a poor retention in reverse-phase liquid chromatography (i.e. polar compounds) [5].

The UHPLC and UHPSFC methods are both valid in the dosing range required by E.M.A recommendation, i.e. 80 – 120 % of the targeted concentration [6]. The SFC showed lower intermediate precision and accuracy than UHPLC. UHPSFC required apolar diluent injection solvent in order to reach a sample composition close to the mobile phase to get a good peak shape. Unfortunately, these solvents (propan-2-ol, hexane, n-heptane) are volatile and then induce more variability than the methanol-water mixture generally used for LC analysis. Nevertheless, UHPSFC method is valid at least in the 80 – 120% of the target concentration interval with acceptance limits set at ± 5%, it demonstrates its suitability for the quality control of manufactured medicines. The successful validation of SFC method for the quantification of amoxicilline is the demonstration of the potential of this technique for quantitative analysis.

Finally, the UHPSFC and UHPLC methods were used for the analyses of real samples. The results given by both methods were similar and led to the same conclusion about non-conformity, showing the interest of SFC outside the academia research context. SFC is clearly a very promising technique in the framework of quantitative analyses for the quality control of medicines.

References

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