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Improvement of a stability-indicating method by Quality-by-Design versus Quality-by-Testing: A case of a learning process

C. Hubert*, P. Lebrun, S. Houari, E. Ziemons, E. Rozet, Ph. Hubert

University of Liege (ULg), Department of Pharmacy, CIRM, Laboratory of Analytical Chemistry, CHU, B36, B-4000 Liege, Belgium

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ABSTRACT

The understanding of the method is a major concern when developing a stability-indicating method and even more so when dealing with impurity assays from complex matrices. In the presented case study, a Quality-by-Design approach was applied in order to optimize a routinely used method. An analytical issue occurring at the last stage of a long-term stability study involving unexpected impurities perturbing the monitoring of characterized impurities needed to be resolved. A compliant Quality-by-Design (QbD) methodology based on a Design of Experiments (DoE) approach was evaluated within the framework of a Liquid Chromatography (LC) method. This approach allows the investigation of Critical Process Parameters (CPPs), which have an impact on Critical Quality Attributes (CQAs) and, consequently, on LC selectivity. Using polynomial regression response modeling as well as Monte Carlo simulations for error propagation, Design Space (DS) was computed in order to determine robust working conditions for the developed stability-indicating method. This QbD compliant development was conducted in two phases allowing the use of the Design Space knowledge acquired during the first phase to define the experimental domain of the second phase, which constitutes a learning process. The selected working condition was then fully validated using accuracy profiles based on statistical tolerance intervals in order to evaluate the reliability of the results generated by this LC/ESI-MS stability-indicating method.

A comparison was made between the traditional Quality-by-Testing (QbT) approach and the QbD strategy, highlighting the benefit of this QbD strategy in the case of an unexpected impurities issue. On this basis, the advantages of a systematic use of the QbD methodology were discussed.

1. Introduction

Development of a quantitative method for impurity assay in a pharmaceutical form (PF) requires an in-depth understanding of the method. In this context, the analytical procedure must take a stability-indicating approach in order to allow a selective determination of related substances (i.e. intermediate and by-product impurities as well as degradation products) as required by pharmaceutical guidelines [1–3]. Indeed, the development of stability-indicating methods is a major concern in the pharmaceutical industry [4]. Nowadays, development of chromatographic methods is largely performed by the traditional Quality-by-Testing (QbT) methodology or the trial-and-error approach. However, such development does not provide neither the ability opportunity to advisedly assess the robustness throughout the development process, nor the possibility of carrying out quality risk management. Both concepts are requirements stipulated in recent U.S. Pharmacopeia (USP) recommendations [5] as well as in pharmaceutical guidelines [6–8].

As recently stated by Nethercote [9] and Orlandini [10], the Quality-by-Design (QbD) concept allows a systematic and scientific approach to the development of analytical methods, enabling an earlier understanding and identification of variables affecting method performance [11–14]. This approach also allows for the implementation of a control strategy based on the enhanced understanding acquired regarding the analytical method. According to the ICH guideline Q8(R2) [6], the QbD approach can be defined as an optimization strategy combining Design of Experiments (DoE) and Design Space (DS). Within the specific context of Liquid Chromatography (LC), QbD is used to simultaneously optimize both the separation process and the robustness of the method over the whole experimental domain (i.e. the knowledge space). A key component of the QbD paradigm is the definition of the DS of analytical methods where assurance of quality is provided [15,16]. In the ICH guideline Q8(R2) [6], DS is defined as "*the multidimensional combination and interaction of input variables* (e.g. *material attributes*) *and process parameters that have been demonstrated to provide assurance of quality*". This guideline also states that "*working within the design space is not considered as a change. Movement out of the design space*

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* Corresponding author. Tel.: +32 4 366 43 19; fax: +32 4 366 43 17.
E-mail address: chubert@ulg.ac.be (C. Hubert).

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is considered to be a change and would normally initiate a regulatory post approval change process". As already discussed in the scientific literature [17–23], the DS consequently defines a zone of robustness, as no significant changes in terms of separation quality should be observed in the resulting chromatograms.

The goal of the present study is to demonstrate the usefulness of the QbD approach within the framework of a real example illustrating an issue with unexpected impurities appearing during the last stage of a long-term stability study of a controlled released drug formulation (under confidential agreement). The QbT approach, used during the initial development of this method, did not provide sufficient knowledge in order to facilitate adjustments to the method and overcome this issue. The characterized impurities (referred to as the impurities), involved in our case study, were determined from the knowledge of the route of synthesis and from active pharmaceutical ingredient (API) stress-test experiments. P4AX91 (Molecular Weight (MW): 102 $\mathrm{g\,mol^{-1}}$), P4FX98 (MW: 115 $\mathrm{g\,mol^{-1}}$), P4NX99 (MW: 116 $\mathrm{g\,mol^{-1}}$), P4SX95 (MW: 139 $\mathrm{g\,mol^{-1}}$) and P4SX92 (MW: 170 $\mathrm{g\,mol^{-1}}$) are all degradation products of P4MX01 (MW: 250 $\mathrm{g\,mol^{-1}}$), the API of the pharmaceutical form. In the particular case in this study, where specific detection at very low concentration was required for all impurities, a Liquid Chromatography coupled to Mass Spectrometry (LC–MS) technique was selected. However, the use of LC–MS for a quantitative analysis of multiple molecules with (very) low molecular weight and extracted from complex matrices (i.e. controlled released formulations) leads to some key issues. Principal among these are analyzer resolution and accuracy, and signal noise and signal spikes in Total Ion Current (TIC) mode. Regarding small molecules extracted from complex matrices, the possibility cannot be excluded that unexpected compounds will appear with pharmaceutical form aging and lead to co-elution and co-detection in a specific Selected Ion Monitoring (SIM) chromatogram when a simple quadrupole mass detector is used. This is even more complex when targeted impurities are structural analogs with a very similar molecular weight (i.e. separated by only one molecular weight unit) and containing heteroatoms. Indeed, in this case study, P4FX98 is a structural analog of P4NX99 and its MW is smaller by only one mass unit. Due to the presence of nitrogen atoms within the structure of these two compounds, the specificity of a simple quadrupole mass detector was found to be insufficient, resulting in an interference of P4FX98 in the P4NX99 SIM chromatogram. The advantage usually obtained through the use of a triple quadrupole mass detector did not apply in this case, as ions obtained from the fragmentation of pseudo-molecular ions of targeted impurities led to the production of daughter ions with the same mass-to-charge ratio (m/z). Therefore, in the presented case study, it was necessary to use a liquid chromatography procedure in order to attain selectivity. An overall methodology was then proposed and discussed. Finally, the selected working condition was fully validated.

2. Experimental

2.1. Chemicals and reagents

Methanol (HPLC gradient grade) was purchased from J.T. Baker (Deventer, the Netherlands). Water (ULC/MS grade) and acetonitrile (HPLC supra-gradient grade) were provided by Biosolve B.V. (Valkenswaard, the Netherlands). Formic acid (98–100%, analytical grade) and ammonia solution (32%, extra pure) were acquired from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q Plus 185 water purification system from Millipore (Billerica, MA, USA). Impurities, the API and P4NX99-D, the P4NX99 molecule labeled with deuterium employed as the internal standard (IS), were all kindly provided by the supporting pharmaceutical company.

2.2. Sample preparation

Preparations of stock solutions of each single impurity and the API were divided into three groups. This was done in order to prevent any peak distortion such as a retention time shift or peak fronting and/or tailing due to different elution strengths between the injected solution and the tested mobile phase. Group 1 was prepared using methanol (MeOH) as the organic modifier. Solutions in Group 2 were made using acetonitrile (ACN), while in Group 3 an equal proportion of methanol and acetonitrile (MeOH/ACN) was used. Therefore, stock solutions were prepared three times (i.e. one for each Group) by dissolving an appropriate quantity of analytes in the dissolving solvent, a mixture of formic acid 0.1% and organic modifier in the proportion 80/20 (v/v). The organic modifier was selected accordingly with the Group description made above. All stock solutions were prepared daily and were diluted using a dissolving solvent containing the organic modifier in the proportion of the tested mobile phase to reach a concentration of 5 $\mu\mathrm{g\,mL^{-1}}$ for each impurity and for the API. The relatively high concentration of these solutions was selected to ensure detection despite the large number of ions simultaneously being recorded during the whole run time (i.e. all impurities and the API).

Aged tablets (the pharmaceutical form involved in this study) and aged placebo tablets (i.e. tablets from completed long term stability studies (36 months) and completed accelerated stability studies (12 months) [1]) were kindly provided by the supporting pharmaceutical company. Preparation of the pharmaceutical form was performed by a generic extraction protocol. A validation step was performed on the two compounds of the critical pair of monitored impurities, P4NX99 and P4FX98 in the presence of all the other impurities and the API (see Supplementary data document). During the validation phase, the calibration standards within the matrix and the validation standards were both prepared taking into account placebo tablets and an appropriate quantity of P4MX01 (i.e. the quantity corresponding to the mass of API contained in four tablets) in order to mimic real samples. The P4MX01 used in this section of the study was a pure chemical free of its degradation products.

2.3. Experiments

Experiments were performed on LC/MS equipment composed of: for the chromatographic part, a Waters (Milford, MA, USA) sample manager 2777, a CTC Analytics AG (Zwingen, Switzerland) Stack Cooler DW with a CTC Analytics AG Peltier thermostat allowing cooling of samples at 10 °C, four Waters binary HPLC pumps 1525 μ, and a Waters temperature control module controlling a columns oven. The MS detector consisted of a Waters Micromass single quadrupole (Quattro, Ultima/ZQ) equipped with a Micromass 4-way Multiplexing Interface (MUX) in order to allow a 4-way analysis if necessary (Fig. 1). LC/MS parameters that were not investigated during the development process (apart from the columns description) are described in Table 1.

2.4. Optimization study

The optimization study was designed in two phases: (i) optimization of the method for the determination of impurities and the API, (ii) optimization of the method taking into account the aged matrix, which can contain unexpected degradation products leading to multiple peaks in a specific SIM chromatogram (i.e. SIM chromatograms of impurities). During the optimization phase, the responses evaluated were the retention times when the principal