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Development and validation of an UPLC method for determination of content uniformity in low-dose solid drugs products using the design space approach

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ABSTRACT

A simple and reproducible UPLC method was developed and validated for the quantitative analysis of finasteride in low-dose drug products. Method validation demonstrated the reliability and consistency of analytical results. Due to the regulatory requirements of pharmaceutical analysis in particular, evaluation of robustness is vital to predict how small variations in operating conditions affect the responses. Response surface methodology as an optimization technique was used to evaluate the robustness. For this, a central composite design was implemented around the nominal conditions. Statistical treatment of the responses (retention factor and drug concentrations expressed as percentage of label claim) showed that methanol content in mobile-phase and flow rate were the most influential factors. In the optimization process, the compromise decision support problem (cDSP) strategy was used. Construction of the robust domain from response-surfaces provided tolerance windows for the factors affecting the effectiveness of the method. The specified limits for the USP uniformity of dosage units assay (98.5–101.5%) and the purely experimental variations based on the repeatability test for center points (nominal conditions repetitions) were used as criteria to establish the tolerance windows, which allowed definition design space (DS) of analytical method. Thus, the acceptance criteria values (AV) proposed by the USP-uniformity of assay only depend on the sampling error. If the variation in the responses corresponded to approximately twice the repeatability standard deviation, individual values for parcentage label claim (%LC) response may lie outside the specified limits; this implies the data are not centered between the specified limits, and that this term plus the sampling error affects the AV value. To avoid this fact, the limits specified by the Uniformity of Dosage Form assay (i.e., 98.5–101.5%) must be taken into consideration to fix the tolerance windows for each factor. All these results were verified by the Monte Carlo simulation.

In conclusion, the level of variability for different factors must be calculated for each case, and not arbitrary way, provided a variation is found higher than the repeatability for center points and secondly, the %LC response must lie inside the specified limits i.e., 98.5–101.5%. If not the UPLC method must be re-developed.

1. Introduction

Finasteride is a 4-aza-3-oxosteroidal inhibitor of human 5α-reductase. It is a member of the family of compounds referred to as 4-azasteroids that block the intracellular metabolism of testosterone and thereby enable the more potent androgen dihydrotestosterone to come into play [1,2]. Chemotherapy with finasteride has shown a beneficial effect in the prevention of prostate cancer, which is the most common cancer among men over 50 years with increasing prevalence with age [3]. At present, finasteride is marketed in low-dose dosage form (1–5%). The analysis of high-potency, low-strength solid oral dosage forms poses a number of analytical challenges regarding potency, purity and dissolution testing of the dosage form. The low quantity of active pharmaceutical ingredient (API) and its corresponding degradation products in these dosage forms results in sample solutions with extremely low analyte concentrations that pose difficulties for detection and quantitation. The high excipient-to-drug ratio in low-dose products poses additional challenges such as difficulties in extracting the entire active ingredient, leading to low potency (i.e. the amount found is lower than the label claim) or irreproducible assay results. Potency and purity results can also be affected by interferences from the excipient or excipient-related impurities.

At present, the quality control of API in formulations from the pharmaceutical industry has been largely based on well-established and officially recognized HPLC methods. However,

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HPLC analysis time and resolution are limited by particle size and instrumentation. Ultra-performance liquid chromatography (UPLC) technique, commercially available from 2004 [4], offer efficient chromatography with reduced run times and improved sensitivity [4,5] by taking advantage of smaller particle size (1.7 μm) and higher operating pressures than conventional HPLC. The additional benefit is the significantly reduced consumption of mobile phase compared with HPLC. Owing to its speed, sensitivity, and lower waste and cost of performing an analysis, this technique has been gaining considerable attention in recent years including the pharmaceutical analysis [6].

Method validation is a procedure to confirm that the analytical method applied in a specific test is suitable for its intended use. Results from method validation can be used to determine the reliability and consistency of analytical data, but a fundamental criterion of quality is robustness. The ICH-Q2-(R1) guidelines clearly defines robustness [7]. It should be tested before method validation to avoid undesired surprises, costly repetitions, and the method having to be re-developed and re-optimized [8,9]. Robustness has to be studied by applying changes in operating conditions within the same order of magnitude as those which could occur by chance when running the method routinely. The design of experiments (DOE) method provides an effective, efficient approach to evaluate simultaneously the effects of factors and their interactions, and to model and predict the relationship between these factors and the responses with a limited number of runs [10].

Since the adoption of the ICH Q8 [11] document concerning the development of pharmaceutical process following a Quality by Design (QbD) approach, there have been many discussions on the opportunity for analytical method developments to follow a similar approach. A key component of the QbD paradigm is the definition of the Design Space (DS) of analytical methods where assurance of quality is provided. The DS requirement of the ICH Q8 [11] states that the DS is a region where process parameters "have been demonstrated to provide assurance of quality". i.e., the DS allows determining the critical analytical method parameters and their respective range of variation. This implies that the DS of an analytical method is a measure of its robustness. Additionally, as moving within the DS is not consi&hyphen-qj;dered a change, more flexibility for the analytical methods during its routine application is possible. Hence change controls will only be required when stepping outside the DS limits [12]. Response-surface designs are key tools to define the DS of analytical methods. They study a large experimental domain, the behavior of the responses with respect to the studied factors, and they provide a model to predict the value of the response within the range of these levels of factors [12].

The aim of this study was to solve the difficulties encountered while developing a single UPLC method for a fixed combination product where the API is present at a low dose with respect to the excipients. Specifically, this paper presents the robustness study of the UPLC method for the quantitative determination of finasteride using the following analytical strategy based on response surface methodology and establish the DS of analytical method: (i) the selection of a statistical design to investigate the experimental region of interest; (ii) perform the experiments in random order; (iii) perform analysis of variance (ANOVA) on the regression results so that the most appropriate model with no evidence of lack of fit can be used to data analysis and, simultaneously, identify the factors and interaction effects which potentially affect the responses; (iv) validate the obtained model in order to evaluate whether the system is really optimized or not; in the optimization process, the cDSP strategy was used; and (v) define a robust domain from the response-surfaces in order to determine the tol&hyphen-qj;erance windows for the factors. For this, the level of the analytical method variability required was established in accordance with the specification limits for the total dose and uniformity of dosage unit [13]. Finally, a Monte Carlo simulation method was used to check results. All these aspects were analysed using commercial finasteride tablets and finasteride-lactose mix prior to filling the capsule as model.

Table 1
Factors and coded levels used for evaluation of the robustness in accordance with the central composite design. The experiments were randomized but with the constraints to perform center points at regular intervals.

Runorder	Factors			Levels			Response	
	T(°C)	F(mL/min)	Me(%)	T	F	Me	k′	%LC
16	37	0.37	62	−1	−1	−1	1,377	1,087
11	43	0.37	62	1	−1	−1	1,203	1,092
5	37	0.43	62	−1	1	−1	1,382	0,934
6	43	0.43	62	1	1	−1	1,198	0,940
18	37	0.37	68	−1	−1	1	0,633	1,061
15	43	0.37	68	1	−1	1	0,546	1,067
3	37	0.43	68	−1	1	1	0,633	0,911
7	43	0.43	68	1	1	1	0,563	0,913
8	35	0.40	65	−1,682	0	0	0,976	0,991
19	45	0.40	65	1,682	0	0	0,772	1,018
10	40	0.35	65	0	−1,682	0	0,878	1,137
2	40	0.45	65	0	1,682	0	0,873	0,882
13	40	0.40	60	0	0	−1,682	1,685	1,010
14	40	0.40	70	0	0	1,682	0,450	0,979
1	40	0.40	65	0	0	0	0,880	0,986
4	40	0.40	65	0	0	0	0,882	0,988
9	40	0.40	65	0	0	0	0,889	0,987
12	40	0.40	65	0	0	0	0,870	0,984
17	40	0.40	65	0	0	0	0,873	0,991
20	40	0.40	65	0	0	0	0,871	0,983

2. Experimental

2.1. Materials

Finasteride (Lot No. 102857) and lactose monohydrate (Lot No. 091973) were purchased from Acofarma (Barcelona, Spain). The finasteride tablet for oral administration used in this study was supplied by MSD Ltd. (United Kingdom). The composition per tablet is: Finasteride (1 mg), lactose monohydrate (110,4 mg), microcrystalline cellulose, corn starch, talc, and magnesium stearate. Ethanol and methanol (HPLC grade) were from Merck (Darmstadt, Germany). Deionized water was purified in a MilliQ plus system from Millipore (Molsheim, France).

2.2. UPLC system

Analytical separations were performed with an ACQUITY™ UPLC system equipped with a micro-vacuum degasser, thermostatted auto-sampler, binary gradient pumps, thermostatted column compartment, tunable UV detector, and an ACQUITY™ UPLC BEH C18 column (50 × 2.1 mm, 1.7 μm), all obtained from Waters Corp.(Milford, MA, USA). The column temperature was maintained at 40 °C. An isocratic mobile phase consisting of a 65:35 (v/v) mixture of methanol and water was used, which was prepared with the pump from pure solvents, at flow rate of 0.4 mL/min. The autosampler temperature was kept at 20 °C and the detection monitored at a wavelength of 225 nm. The injection volume was 5 μL. The data were collected and processed using Empower™ software (Waters Corp.).