Talanta 115 (2013) 490–499

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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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ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form
21 May 2013
Accepted 28 May 2013
Available online 18 June 2013

Keywords:
UPLC
Robustness
Response surface
Repeatability
Design space
Finasteride

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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http://dx.doi.org/10.1016/j.talanta.2013.05.060

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 considered 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 tolerance 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.).

2.3. Robustness study: design of experiments

Three factors were analyzed in the robustness study: column temperature (*T*), flow rate (F) and percentage of methanol in mobile phase (Me). However, the choice of variations to be applied

to the factors is always difficult in a robustness study, because the variation ranges have to satisfy two inconsistent criteria. Indeed, such variations have to reflect small variations that may occur by chance when the method is transferred, e.g. when the analysis is made by another operator or with other equipment. Moreover, the variations have to be great enough to be applied in a reliable way (see Table 1).

Since a robustness study must describe the response surface around the nominal conditions, at least second-degree modeling is necessary. In this case, the second order interactions like Me × T, F × T or T × T could be potentially influential, so the following equation was used:

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i<1}^{3} \sum_{j=2}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \quad (1)$$

where y is the response, β_0 the intercept, β_i the main coefficients, β_{ij}, the two-factor interaction coefficients, and β_{ii} the quadratic coefficients. The responses used for the data analysis were retention factors (k′) for the identification of drug and concentration for their quantitation.

A circumscribed three-factor central composite design (CCCD) with five coded levels such as −1.682, −1.0, 0, +1,and +1.682, was used where six central point repetitions were carried out to find the experimental error variance and the validity of the model. Thus, 20 experiments were performed (Table 1).

All experiments were randomized to minimize the effects of uncontrolled factors that could affect the final results, except for sampling the center points at regular intervals as well as at the beginning and end of the study to detect any problem with the column (Table 1).

The experiments began when the chemical equilibrium was reached, at least10 column volumes.

2.4. Method validation

The developed UPLC method was validated in terms of specificity, linearity, accuracy, precision, robustness and stability according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use (ICH) guidelines [7].

System suitability parameters were measured so as to verify the performance of the system. All important characteristics were measured, including retention and tailing factors, and theoretical plate number. System precision expressed as repeatability was determined on six replicate injections of standard preparations. The intermediate precision of the assay was also evaluated by the same analyst on three different days. The accuracy of the assay method was evaluated in triplicate using three concentration levels 2, 10 and 20 μg/mL. Both parameters were examined by the relative standard deviation (RSD) of recovery data.

Linearity of response was assessed by injecting standards prepared by serial dilution of stock solution, using the mobile phase as diluent. The peak area of standard compounds was plotted against the respective concentrations. The linear regression method was used for data evaluation. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by the signal to noise approach as defined in the ICH guideline [7].

To assess the proposed UPLC method as a stability-indicating procedure for finasteride, chromatograms (see Fig. 1) were recorded under various stress conditions where degradation was stimulated by heat and light.

2.5. Blend preparation/milling of drug substance

The blend was prepared with a ratio of finasteride-lactose (1:199 mg) per unit dose, and milled using a high shear paddle laboratory mixer (Unguator B/R® mix system, Microcaya Co., Spain) for 3 min. at 300 rpm. Two batches were prepared at laboratory scale.

2.6. Assay of pharmaceutical preparations

Samples of 200 mg of drug blend were accurately weighed and transferred to a 25-mL volumetric flask with a mixture of ethanol/water (50/50, v/v). The content was filtered through a Whatman® filter paper (Whatman Int., England). A portion of this solution was diluted with mobile phase to obtain concentration values within the calibration range, then finally, the sample was filtered through a 0.45 μm filter (Nylon Acrodisc, 25 mm) before injecting- 5 μL sample into the UPLC system. The same procedure was followed

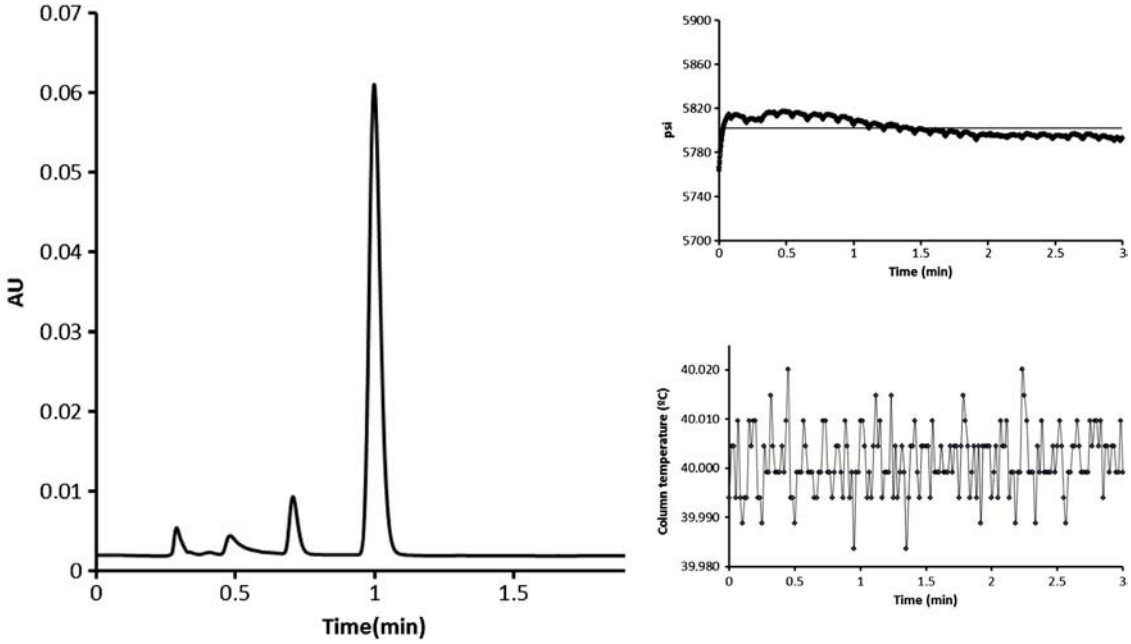

Fig. 1. UPLC chromatograms obtained from the analysis of powdered tablets of finasteride and the control chart for the system backpressure and column temperature obtained from Empower® software (Waters).

for the content uniformity test, using one tablet per sample. In this case, each tablet was weighed and finely pulverized beforehand.

2.7. USP (905) uniformity of dosage units

The uniformity of the dosage unit (UDU) is usually a product specification for oral solid dosage forms and is tested to ensure it meets the compendial acceptance criteria. For this, the criteria from United States Pharmacopeia <905> Uniformity of Dosage Form [13] harmonized with European Pharmacopoeia 2.9.40 [14] are based on a two-stage process using acceptance criteria values (AV).

For content uniformity, 10 dosage units are examined first to obtain the Stage-1 AV. If the AV exceeds the criterion, the first test is failed and the process will enter Stage-2 by examining 20 additional units. If the Stage-2 AV and the first and last of the 30 units comply with the criteria, the test passes. Details of the passing criteria are to be found in the bibliography [13,14]. In order to pass Stage 1, the mean and standard deviation of the samples must fall, schematically, within a trapezoidal area defined by the criteria. All the measurements of dosage units and criteria values are in the percentage label claim.

3. Results and discussion

3.1. Method development

Several preliminary studies were carried out to identify the variables with major effects on the system and to estimate the range and the magnitude of variation of each factor in order to obtain the DS of the analytical method. In our case, the variables were: flow rate, column temperature and methanol content in the mobile phase. In this point, screening studies are very useful to determine which of the several experimental variables and their interactions present more significant effects [8], but this suppose an increase in the consumption of reagents, materials and, especially, time. For this, taking into consideration the objective of the study and the experience of the researchers, finasteride was adequately separated using 65% methanol in the mobile phase, and a column temperature of 40 °C. The retention factor was slightly lower than 1 (k′=0.9), while the flow rate of 0.4 mL/min was optimized with regard to the backpressure, column life, and analysis time as well. At this flow rate, a backpressure of 5800 psi was observed, and the runtime was established at 2 min, although this value can be lowered to 1.5 min without affecting separation. These conditions involve a compromise between all chromatographic factors and UPLC equipment.

3.2. Robustness evaluation

3.2.1. Data analysis

Response surface methodology as an optimization technique was used to evaluate the robustness of the analytical method [9]. After the experimental region has been delimited, the following steps were the choice of the experimental design and carrying out the experiments according to the selected experimental matrix and, the evaluation of the model's fitness using the appropriate statistical analysis. For this, a circumscribed three-factor central composite design (CCCD) with five coded levels and six central points was applied to estimate the effect of three factors on retention factor (k′) and drug concentration expressed as percentage label claim (%LC). These two responses defined as critical quality attributes (CQAs) [12] are used to judge the quality of the analytical method developed.

The coefficients of the polynomial model given by Eq. (1) were estimated by multiple linear regressions whereas the identification of significant coefficients was carried out by ANOVA [15]. To test the significance of the model's lack-of-fit, the ratio of mean square for lack-of-fit to the mean square for pure error was also computed (Table 2).

The adequacy of the model was evaluated using the lack-of-fit test for both responses. If the lack of fit was not significant, the total error was used to evaluate if the model coefficients were significant through a Student-*t*' test (Table 3). To determine the relative effect of each factor on the response (% Effect), the method proposed by Destandau et al. [16] was used.

In the case of the retention factor (k′), the F-test result shows that the $F_{observed}$ value was lower than the F_{tabled} one for $\alpha=0.05$, i.e. the probability is higher than 0.05 ($p > 0.05$), thus the lack of fit was not significant for this response. From the ANOVA, it is clear that the main terms (T and Me) and the second-term (T × Me and Me × Me) contribute significantly to the model. The flow rate under factor or interaction form was never found to be influential. Methanol content was the most significant factor, especially as a main factor with an effect of around 40%, whereas *T* was significant, but with a smaller effect around of 7.3%. The canonical analysis indicates that the stationary point of the fitted surface is fairly distant from the experimental region (in coded units it is situated at 0.75, 3.68, and 2.46, which correspond to: *T*=42.2 °C; F=0.509 mL/min; and Me=72.3%; these latter two are out of the studied region), and the eigenvalues are of mixed sign, indicating it is a saddle point (neither a maximum nor a minimum). If the purpose is to obtain a maximum or minimum response to a

Table 2
ANOVA results for the response surface quadratic model fitted to the central composite design results reported in Table 2.

Factors[a]	k′				%LC		
	Df	Sum Sq	Mean Sq	F value	Sum Sq	Mean Sq	*F* value
Response							
F0	3	1.78486	0.59495	3591.9	808.93	269.6	1214.9
TWI	3	0.00509	0.00170	10.25	0.02	$8.1 \cdot 10^{-3}$	0.0357*
PQ& SO	3	0.06631	0.02210	133.4	11.71	3.905	17.59
Residuals	10	0.00166	$1.7 \cdot 10^{-4}$		2.22	0.222	
Lack of fit	5	0.00138	$2.8 \cdot 10^{-4}$	4.97*	1.80	0.361	4.35*
Pure error	5	0.00028	$6 \cdot 10^{-5}$		0.41	0.083	

* P > F.
[a] FO, TWI, PQ and SO correspond to the "first-order", "two-way interaction", "pure quadratic" and "second-order" terms, respectively.

Table 3
Evaluation of the factors significativity and their effects on the responses, retention factor(k′) and finasteride percentage label claim (%LC) assuming a second-degree model. Only significant coefficients are given.

Term	k′			%LC		
	Estimate	P > |t|	Effect (%)[a]	Estimate	P > |t|	Effect (%)
Intercept	0.8775	< 0.0001		98.660	< 0.0001	
T	−0.06283	< 0.0001	6.82	0.4716	0.0041	0.47
F				−7.5991	< 0.0001	7.62
Me	−0.3560	< 0.0001	38.6	−1.1212	< 0.0001	1.13
T × Me	0.02512	0.00025	2.72			
T × T				0.5688	0.0010	0.56
T × F				0.7456	0.00013	0.76
Me × Me	0.06698	< 0.0001	7.27			

[a] The relative effect (as a percentage) was calculated by dividing the coefficient estimate by the mean of the responses in accordance with [16].

studied system, the saddle point coordinates do not serve as optimal values.

The same analysis was performed on the finasteride %LC response. The lack of fit was also non-significant ($p > 0.05$), so the total error was used to evaluate if the model coefficients were significant (Table 2). The three main factors were influential; the most was flow rate with an effect of 7.6%. Among the second order terms the quadratic terms T × T and F × F were significant, but with similar effects to those observed for T and Me as main factors (Table 3). In the canonical analysis, the stationary point of the fitted surface was again fairly far the experimental region (the coded unit is situated at −0.17, 5.07, 2.44, which correspond to: T=39.5 °C; F=0.550 mL/min; Me=72.2%; these latter two were outside the studied region), and the eigenvalues were positive, indicating it is a minimum. This situation leads to an undesirable response, especially for k. A predicted k value of 0.40 was obtained, whereas for %LC response was 0.81 and that it is necessary to displace (if possible) the initial design to attain a fully desirable response (or CQA) meetings pre-defined quality criteria.

3.2.2. Optimization

Murphy et al. [17] reviewed multi-response optimization techniques which, for our purpose, can be classified in two broad groups. The first group encompasses those methods based on the construction of a desirability function, the simplest form being the weighting sum of the response variables. This kind of method assumes it is worth accepting a trade-off between the response variables. The second group involves those methods based on the so-called compromise decision support problem (cDSP). This aims to minimize the difference between the goal and the actual performance, namely, the deviation function. There are two alternatives to prioritize the system goals: Archimedean and lexicographic. The Archimedean weighting scheme assigns explicit weights to each system response. The lexicographic alternative is based on an ordered minimization scheme; the first response variables to be optimized are those with higher priority; optimization of the remaining variables is carried out sequentially, from the highest to the lowest priority.

The optimization of chromatographic analytical methods is a multi-response optimization problem in which the response variables can be ordered according to the priority assigned to each variable. The higher priority response variable is the analytical error measured through the precision or intermediate precision as discussed later on. Lower priority response variables are those related to the quality of peak separation and integration. In both cases, we must set-up a maximum difference allowable from target values in order to maintain the quality of the analytical method, and therefore we must optimize the method following the compromise support problem strategy.

The purpose of the analytical method developed in this paper is to assess drug content uniformity according to the harmonized test for uniformity of dosage units (UDU test). It is well recognized that to have a high probability (not less than 0.90) of passing the UDU-test, the variability of drug content of individual dosage units (tablets, capsules), expressed as relative standard deviation (RSD), should not exceed 6.5%. However, the total variance (σ^2) can be split as in [18]:

$$\sigma^2 = \sigma_B^2 + \sigma_T^2 + \sigma_A^2 \quad (2)$$

where ${\sigma_B}^2$ is the variance resulting from the blending process, ${\sigma_T}^2$ the variance from the tableting process and ${\sigma_A}^2$ the variance of the analytical method. It is unclear how much would be ${\sigma_B}^2$+${\sigma_T}^2$; the aforementioned FDA guidelines suggest it would be around 2.76% [19], but in our opinion the interval 2.5%–4.5% would be acceptable. These figures lead us to an interval for ${\sigma_A}^2$ between 2.0 and 4.0% [19].

Let y_i be the amount of drug in the sample i-eme expressed as percentage of label claim as in the UDU-test, and let T be the true value. The expected value of the difference between the observed and the true values is then [20]:

$$E[y_i - T] = (y_i - \mu) + (\mu - T) \quad (3)$$

and,

$$E[(Y-T)^2] = \sigma_A^2 + (\mu - T)^2 \quad (4)$$

A sample estimated from the squared difference between the measurements and the true value is $[y_i-100]^2$. As long as this is an estimate of the variance (${\sigma_A}^2$) plus the square of the skewness of the analytical method $[\mu-T]^2$, it constitutes a suitable high priority response to be optimized. Additionally, we can set up a maximum value in order to find the optimum region for the control variables.

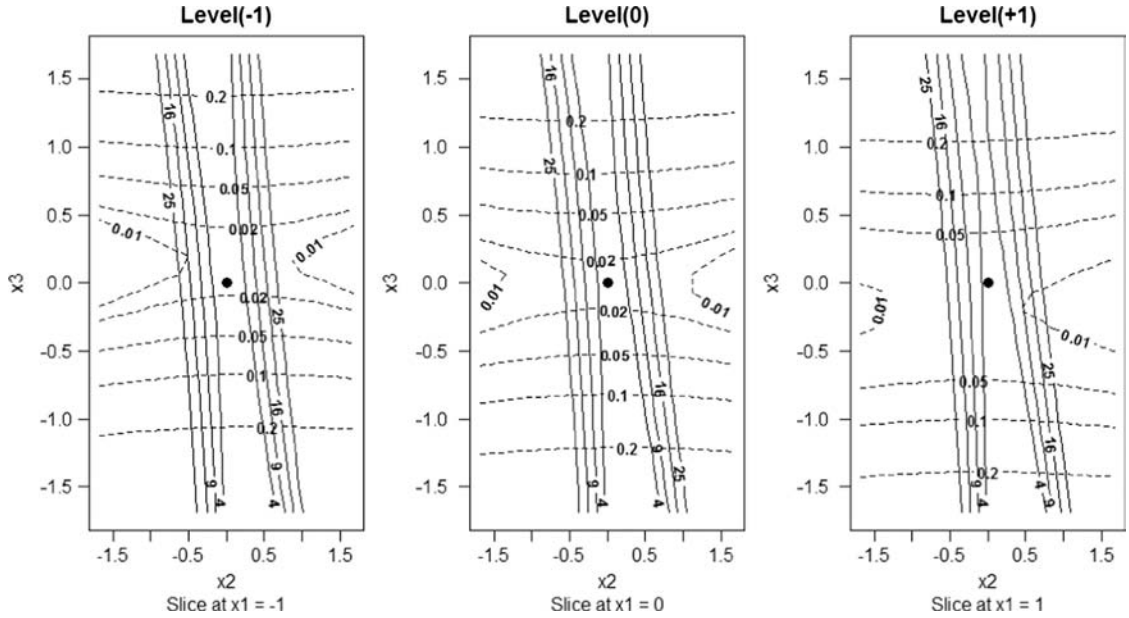

Fig. 2. 2D-Contour levels for primary difference function $[y_i-100]^2$ (continuous line) and secondary difference function $[k'-1]^2$ (dotted line) for the variables column temperature (x1), flow rate (x2) and methanol content (x3) The black points show the nominal conditions.

The secondary variable already proposed in this paper is the retention factor k′; it is required that 0.9≤ k′ ≤1.10, and therefore we proposed as secondary difference function $[k'-1]^2$ with an upper bound value equal to 0.010.

Fig. 2 depicts contour levels in the plane for the variables methanol content (Me) and flow rate (F) for three levels of column temperature variable T (−1, 0 and +1) for the primary response variables, $[y_i-100]^2$ (continuous line), and for the secondary response variable $[k-1]^2$ (dotted line). We have also included the central point of the experimental design. The levels of the curves for the primary response variable are: 2.0%, 3.0%, 4.0% and 5.0% [19].We can conclude that the nominal conditions corresponding to the central point of the experimental design fulfill the validation requirements, even for the most stringent conditions. Moreover, it can also be observed that there is enough margin for some variation in the experimental conditions, keeping the primary response under acceptable values. This itself constitutes an indication of the robustness of the proposed analytical method.

3.2.3. Robust domain definition

At first, given that some coefficients were influential, the tolerance windows had to be established in order to define the robust domain (within which the response will be considered rugged). The procedure described by Destandau et al. [16] was used to define it. As the "finasteride %LC" response was a function of the three factors, the central composite surface should be projected on the two-factor planes, thus defining contour diagrams. In our case, three kinds of contour diagrams were drawn (Fig. 3). The final robust domain was then defined by the intersection of the valid domains found for the analyzed responses. Beyond these robust domains, the response was regarded as rugged.

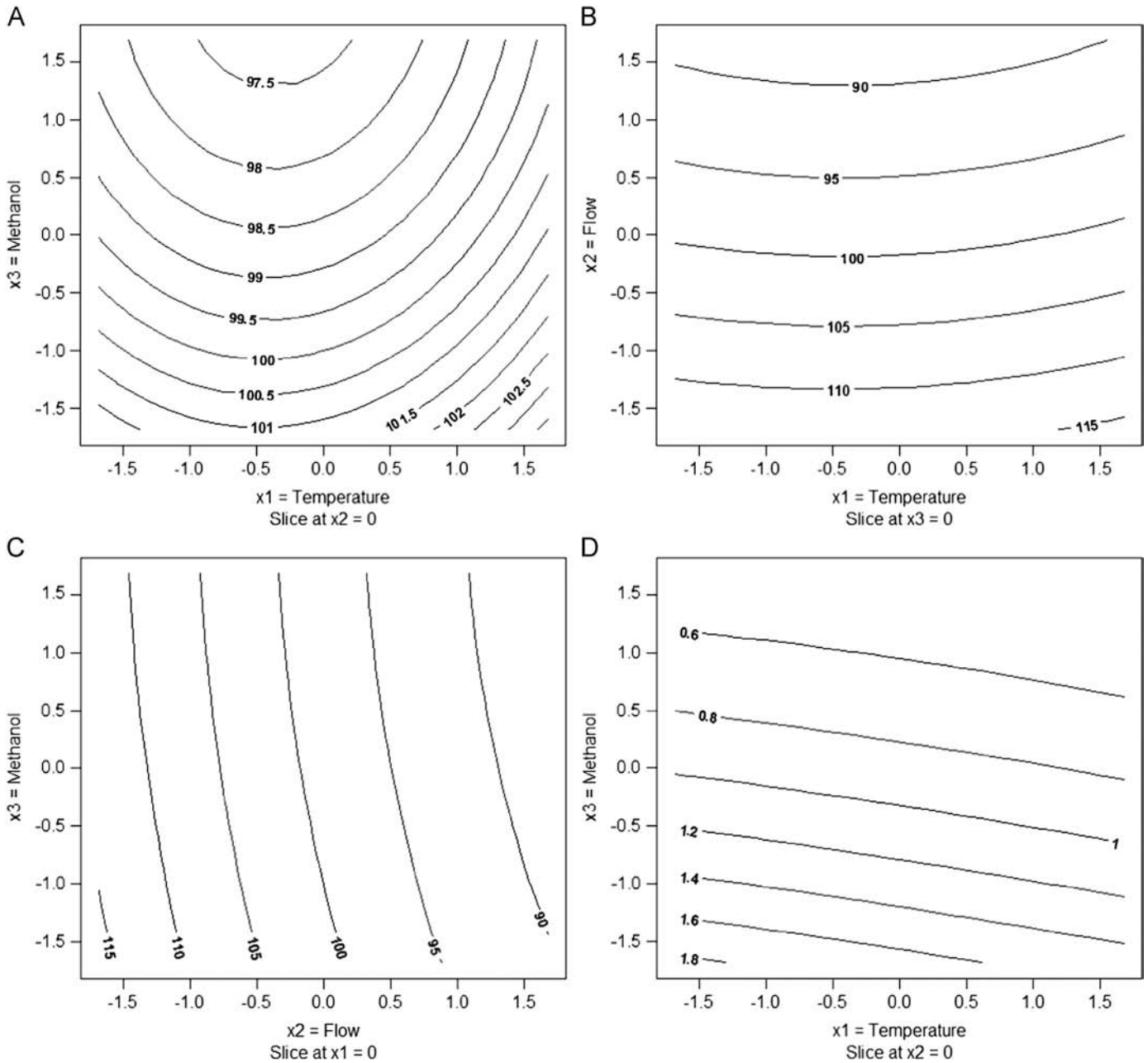

Fig. 3. 2D-contour plot obtained by the circumscribed central composite design for finasteride %LC (A, B, C) and retention factor (D) response in function of column temperature (X_1), flow rate (X_2) and methanol content (X_3). The contour lines show the specified limits by the USP uniformity of dosage unit assay (98.5–101.5%).

From the regulatory prospective, the specification limits for the total dose of finasteride tablets are set at 95–105% of the label claim, and the content uniformity is evaluated for the USP ⟨905⟩ Uniformity of Dosage Form [13]. This guideline uses the acceptance value (AV) shown by Eq. (5) to determine the test passes.

$$AV = |M - \overline{X}| + ks \tag{5}$$

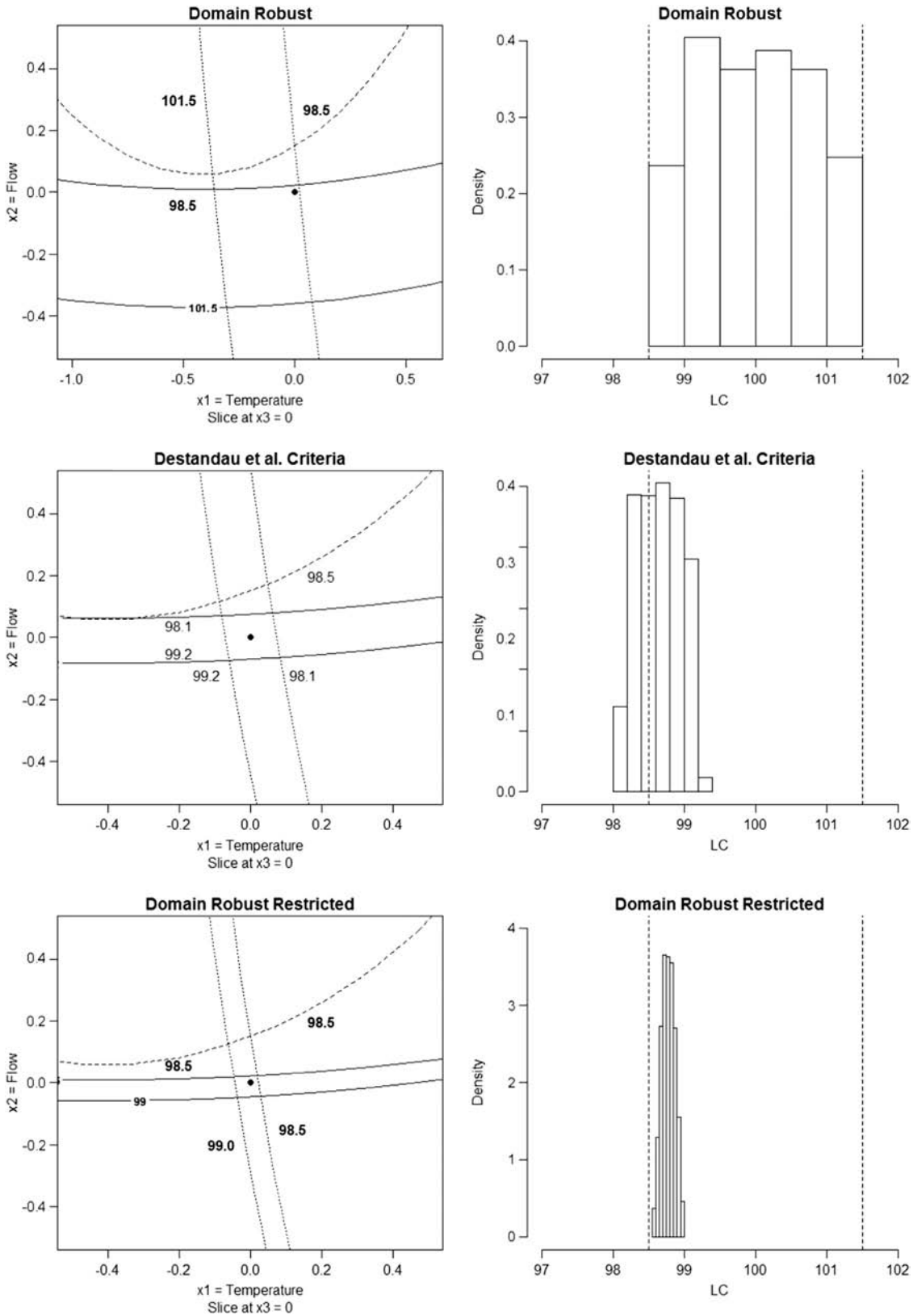

Fig. 4. Robust domain boundaries for finasteride %LC response in accordance with the criterion reported in Table 5 (left column). Histograms of simulated data set ($n=2000$ runs of subgroups of 10) obtained for different case (right column). The dashed line shows the specified limits by the USP uniformity of dosage unit assay (98.5–101.5%).

Table 4
Tolerance windows obtained for three factors (T, F, Me) in function of different rules applied. In all case, the mean values lay inside the specified limits (i.e., 98.5–101.5%) except for the Destandau et al. criteria.

Factor	Nominal doller06#conditions	Domain doller06#robust	Destandau et al. doller06#Criteria	Domainrobust doller06#restricted
T (°C)	40.0	38.9–40.2	39.76–40.25	39.88–40.12
F (mL/min)	0.400	0.388–0.401	0.398–0.402	0.399–0.401
Me (%)	65.0	63.9–65.2	64.8–65.24	64.81–65.19
%LC Limits	98.5–101.5	98.5–101.5%	98.1–99.2%	98.5–99.0%

This guideline considers simultaneously the interplays of the potency mean ($\overline{X}$), the sample standard deviation (s), and the ratio of the target dose to the label claim, i.e., the target content per dosage unit at the time of manufacture, expressed as a percentage of the label claim. Unless otherwise specified in the individual monograph, the target content is the average of the limits specified in the potency definition in the individual monograph. For the finasteride, the target content is equal to 101.5, and thus, reference value (M) is defined as follow: $M=98.5\%$ if $\overline{X}<98.5\%$, $M=101.5\%$ if $\overline{X}>101.5\%$ and $M=\overline{X}$ otherwise.

Thus, AV described by Eq. (5) provides a measure of the dispersion (ks) and of centeredness, i.e. if the data are perfectly centered between the specified limits (M-$\overline{X}$). The choice of the limit 98.5–101.5% requires that the bias (i.e. systematic error) with respect to the $\overline{X}$ value is zero and thus the AV value only depends on the magnitude of sampling error and second, the total variance of the analytical method is included in these specification limits [19]. We therefore propose using these values to establish the tolerance windows for the response. Also these limits allow to fix the DS of analytical method, i.e. the experimental region where process parameters to provide assurance the quality as the ICH-Q8 states [11].

Fig. 4 shows the robust domain boundaries obtained from the superimposition of the contour lines obtained for the tolerance windows applied to the finasteride %LC response, according to the main factors (T, F and Me). This final domain also allowed the tolerance windows to be known for different factors (Table 4). The nominal conditions represented by a circle are included in the confidence domain, although it is far off center, which implies than the tolerance windows are not symmetric with respect to the nominal conditions. To confirm the validity of our results, a Monte Carlo simulation was carried out using the R program [15]. During the sample run, flow rate, column temperature or percentage of methanol in mobile phase can fail, either one, two or all them at the same time; this last situation is less probable. In the Monte Carlo simulation study, we have considered this case, and for this a uniform distribution was assumed. The variables can vary in the range of values defined by the robust domain and the %LC response is calculated. Ten values were taken and the mean and standard deviations were calculated. This process was repeated 2000 times. Fig. 4 shows the histogram of the data ($n=2000$ runs of subgroups of 10 values), and the results showed that all mean lay inside the specification limits, the RSD was lower than 1.5%, whereas the AV value varied between 2.8 and 3.6. If the specification limits for the total dose of finasteride tablets are used, set at 95–105% of the label claim, all individual measurements varied between 98.5% and 101.5%, which implies all values lie within the tolerance window.

However, one should remember that the small but deliberate changes for robustness studies ought to be compared with expected experimental errors. In our case, the tolerance windows shows variations very far from the experimental protocol since the precision level of the new UPLC instruments is lower than 0.05% (see Fig. 1).These results suggest that the robust domain boundaries could be more restricted. For this, the criterion proposed by Destandau et al. [16] and Le Mapihan at al. [21] could be a good alternative. These authors proposed totally arbitrary tolerance windows based on the test repeatability for center points (nominal condition repetitions), where the purely experimental variations are considered to define the domain boundaries. In our case, a value of 0.29% was obtained. Table 4 shows the allowed intervals for each factor considering a variation in the responses corresponded to approximately twice the standard deviation of repeatability, in accordance with these authors. In this situation, individual measurements lower than 98.5% can be obtained for % LC response and thus, the $\overline{X}$ value may lie outside the 98.5–101.5% window (see Fig. 4). This fact implies that the data are not centered between the specified limits (M-$\overline{X}$) and secondly, this term plus the sampling error affects the AV value, but the AV value was always lower than L1, and the test passes. 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 and, then the DS of analytical method. Combining both factor determines that the maximum variation corresponds to the factor with the smallest variability among the three, in our case the flow rate, obtaining a value of 0.30%. This is similar to those obtained in the repeatability test for center points. A Monte Carlo simulation was carried out using these new conditions; the $\overline{X}$ value varied between 98.5–99.0% within the 98.5–101.5% window, the AV < L1, and the test was passed (Fig. 4). These situations supposed to move inside the DS without the need to carry out changes, the analyst have more flexibility during its applications and the information obtained is reliable.

Thus, 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.

In all situations analyzed during the robustness evaluation, the analyte was adequately resolved. The retention factor varied between 0.85–1.1 when the variations in the methanol content of the mobile phase and column temperature were found to be inside the tolerance window. This fact was confirmed from contour diagrams obtained by the intersection of the valid domains found for both responses.

3.3. UPLC method validation

After satisfactory development of the method, it was validated as per ICH guideline [7] to demonstrate suitability for the intended purpose, using the standard procedure to evaluate adequate validation characteristics, i.e., the method should be able to determine assay and impurities of drug in a single run and should be accurate, reproducible, robust, stability indicating, free of interference from degradation products/impurities and straightforward enough for routine use in quality control laboratory.

3.3.1. Specificity

Specificity is the ability of analytical method to measure the analyte response in the presence of its potential impurities, degradation products or others excipients. In dealing with low-

dose drug products, it is important to be aware that adsorption of the drug from the sample solutions onto surfaces can lead to low or variable assay results. These surfaces include filters, volumetric flasks, sample vials/caps and packaging components [22]. Preparing and analyzing control samples using the sample extraction/dissolving solvent can also help determine the source of nondrug-related impurities, which can lead to inaccurate purity assessments and unnecessary investigations, costing time and resources [22].

Placebo samples were analyzed to identify excipient-related peaks and chromatographic interferences during the sample preparation step as above. Interference with the excipients (lactose) was also checked, and none was observed in any of the analyzed cases. Fig. 1 shows a chromatogram obtained from the analysis of powered finasteride tablets that illustrates this.

3.3.2. System suitability

Parameters such as plate count, tailing factor, retention factor and repeatability for peak areas were calculated (Table 5). All were inside the recommended limits by the CDER guidance document except the retention factor [23].

3.3.3. Linearity of response

A standard 1 mg/mL stock solution of finasteride was prepared in methanol. Calibrations standards were prepared at eight different concentration levels ranging from 2 to 20 μg/mL, using the mobile phase as diluent, and analytical runs were performed on different days.

Linear relationships between the signal peak areas of finasteride and the corresponding concentrations were found. The regression equations of the calibration curve, the standard deviation values of the slope and intercept, along with the correlation coefficients are presented in Table 5. The ANOVA of linear regression confirmed the linearity of the method used through the rejection of the null hypothesis of lack-of-fit for a significance level of 0.05 (α=0.05). A test for an intercept significantly different from zero can also be made on this data if there is no significant lack of fit [24]. A Student's *t*-test was performed to determine whether the experimental intercepts of the above regression equations was significantly different from the theoretical zero value. In this last case, the confidence interval of the intercept contained the origin, which denotes that the intercept is not significantly different from zero.

A plot of the reference versus predicted values for the samples of the calibration set reveals the presence of systematic errors (bias) [25]. The slope and intercept of this fit were calculated as 0.999 ± 0.007 and $2.39 \times 10^{-6} \pm 7.65 \times 10^{-2}$, respectively. Two independent *t*-tests with 31 degrees of freedom at 95% confidence level demonstrated that there are no significant differences between the experimental values and the values of slope and intercept for an ideal fit, one and zero, respectively. Thus, the absence of bias was verified for the proposed method.

Table 5
UPLC Method validation results for finasteride.

Parameter	Finasteride
Tailing factor	1.01
Retention factor (k′)	0.9
Column efficiency (N)	11400
Repeatability (%RSD)	0.12
Intermediate precision (%RSD)	2
Linearity	Area vs C(μg/mL)
– Intercept (± SD)	1623 ± 923
– Slope (± SD)	12059 ± 83
– r^{2enen}	0.9995
Accuracy (%Recovery)	100.04
Accuracy (%RSD)	2
LOD (μg/mL)	0.56
LOQ (μg/mL)	1.86
Selectivity	No interference
Stability-48 h (%)	100.1

3.3.4. Accuracy

Accuracy was examined by the RSD of recovery data from a minimum of nine determinations over a minimum of three concentrations levels covering the specified range. The average percentage recoveries were found to be 100.04% with an RSD of 2.00%.

3.3.5. Precision

System precision expressed as repeatability was determined from six replicate injections of a sample at 100% of the test concentration yielding an RSD value of 0.12%. Intermediate precision was studied using the same conditions (analyst, apparatus, identical reagents, and short time interval), but performing the analysis on different days. In this case, the overall precision corresponds to time-different intermediate precision:

$$s^2_{I(T)} = s^2_m + s^2_{\text{day}} \quad (6)$$

where $s_m{}^2$ is the between-measurement variance, i.e., the random error in every measurement under repeatability conditions, and $s_{\text{day}}{}^2$ is the between-day variance. This expression gives us the capacity of the analytical method to repeat the test result under the defined conditions. The data obtained were subjected to an analysis of variance. The two precision estimates were obtained, analyzing three independent samples (concentration = 10 μg/mL) on the same day under repeatability conditions, and this process was performed on 3 days in accordance with the design proposed by Dehouck et al. [26]. In each run, the same sample was measured in triplicate and each day all samples were newly prepared (n=27). From this study, the between- measurements variance $s_m{}^2$ and the between-days variance $s_{\text{day}}{}^2$ were calculated to be $5.35 \cdot 10^{-3}$ and $3.53 \cdot 10^{-2}$ respectively; the intermediate precision being expressed as %RSD of 1.99%, whereas the inter-day differences were non-significance ($p > 0.05\%$).

3.3.6. Limit of detection and limit of quantitation

LOD and LOQ were determined by at 0.56 and 1.86 μg/mL respectively, using the signal to noise approach as defined in the ICH guideline [7].

3.3.7. Stability

To assess the proposed UPLC method as a stability indicating procedure for finasteride, chromatograms were recorded under various stress conditions where degradation was stimulated by heat and light for 48 h. No more than a 0.5% difference with respect to the label claim was observed. The stock solution of the reference sample was considered stable, for at least 5 days.

3.4. Label claim recoveries from finasteride blends and tablets

In a low-dose formulation, typical problems associated with blending are high variability in potency (high RSD) and outliers (stray values) in assays for blending samples or finished products [27,28]. The adequacy of mixing to ensure uniformity in power blends and finished dosage units was used as a criterion to assess the specificity of the proposed method.

The proposed method was evaluated in the assay of commercially available tablets containing 1.0 mg of finasteride (Proscar®). Ten replicate determinations were carried out on an accurately

weighted amount of the pulverized tablets equivalent to 1.0 mg of finasteride. The average percentage label claim was 100.4% and the %RSD was 2.63, the AV value being lower than 15 (L1 = 15) and, therefore, the test passes.

Ten samples of each batch of lactose-finasteride blends were taken at random and the mean of percentage recoveries and RSD were calculated. Values of 100.5 and 101.9% were obtained and the %RSD was 6.34 and 4.90%, respectively. Batch #2 satisfies the test in Stage-1 (AV < 15), whereas in batch #1, the AV value was higher than criterion (15.2 > L1) and the process then enter Stage-2 AV, it is necessary to examine an additional 20 units. Batch #2 passed the test since the AV < L1 and the highest and lowest values of the 30 units comply with the criteria shown in the bibliography [13,14].

This significant within-location RSD in the blend data, especially for the batch #1, could be an indication of one factor or a combination of factors such as poorly blending, sampling error, segregation, aggregates or large particle size of drug substance, or analytical method error. However this last can be ruled out, since the analytical variability of levels (precision and accuracy lower than 2.1%) can be considered appropriate to detect any change in the content of drug substance. Muzzio et al. [29] studied the effect of sampling error and segregation process during the characterization of powder mixtures. The uses of a "thief probe" can introduce large errors in sample composition, possibly leading to misleading results [29], whereas the segregation problems are related to the particle size distribution, which has a profound impact on content uniformity of low-dose solid drug products [30]. This is a research topic already under study in our laboratory.

4. Conclusions

A simple, sensitive and reproducible UPLC method was developed for the quantitative determination of finasteride in low-dose drugs products. No interference was observed from solid dosage forms or from surfaces includes filters, volumetric flasks, sample vials/caps and/or chromatographic apparatus.

Application of response surface methodology in the robustness evaluation allowed obtaining large amount of information from a small number of experiments, identifying the factors and interaction effects which potentially affect the responses and evaluation of the fitted model by using ANOVA. The data clearly show that the circumscribed central composite design was appropriate. The cDSP strategy was used to determine the optimal conditions. The results indicate that the nominal conditions fulfill the validation requirements, even for the most stringent conditions. Construction of the robust domain from response-surfaces provided tolerance windows for the different factors. 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 window and, then the definition of DS. The results indicate that the levels of variability for the three factors could never smaller than the boundaries imposed by the repeatability test for center points and secondly, the %LC response must lie inside the specified limits i.e., 98.5–101.5%. In this situation, the AV value only depends on the sampling error, whereas the error due to the measurements process is controlled. The application of criteria proposed by Destandau et al. [16] can be a good alternative, where the tolerance window for each factor are established as a function of response, which is affected twice by the repeatability test. However, individual measurements outside the 98.5–101.5% window can still be obtained.

The proposed methodology in this work pointed out the necessity of definition and graphical visualization of the Design Space and posterior application using different criteria allowed to find the experimental region where process parameters "have been demonstrated to provide assurance of quality" as the ICH-Q8 states.

Acknowledgments

This research was financed by the Ministerio de Economía y Competitividad from Spain as part of project SAF 2010-17083.

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