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Original Article

IMPLEMENTATION OF QBD APPROACH TO DEVELOP AND VALIDATE ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF DULOXETINE HYDROCHLORIDE AND METHYLCOBALAMIN IN PHARMACEUTICAL DOSAGE FORM BY HPTLC METHOD

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ABSTRACT

Objective: To develop and validate High-Performance Thin Layer Chromatography (HPTLC) analytical method for the determination of Duloxetine Hydrochloride (DUL) and Methylcobalamin (MEC) in the standard mixture and pharmaceutical capsule dosage form by implementing Quality by the Design (QbD) approach.

Methods: The chromatographic separation was performed on aluminium plates precoated by silica gel 60 F-254 using propanol: water: 25% v/v ammonia solution (9:2:1, v/v/v) as a mobile phase which was optimized with the help of a design expert. Densitometric analysis was carried out in the absorbance mode at 280 nm.

Results: Compact spots for Duloxetine HCl and Methylcobalamin were found at retardation factor (R_f) value of 0.77±0.02 and 0.55±0.03, respectively. The linear regression analysis data for the calibration plots showed correlation coefficient 0.9989 and 0.999 with a concentration range of 1200-3600 ng/spot and 60-180 ng/spot for Duloxetine HCl and Methylcobalamin respectively. Limit of detection (LOD) and limit of quantification (LOQ) was found to be 113.39 and 343.62 ng for Duloxetine HCl and 6.68 and 20.24 ng for Methylcobalamin, respectively. The method was validated for precision, accuracy, robustness, LOD and LOQ according to ICH Q2 R1 guidelines.

Conclusion: A new, simple, accurate, and precise HPTLC analytical method has been developed and validated for the determination of Duloxetine HCl and Methylcobalamin in pharmaceutical capsule dosage form by QbD concept in favour of fewer trials and error-free experimentation for the optimization process. The method seems to be suitable for the quality control in the pharmaceutical industry because of its sensitivity, simplicity, and selectivity.

Keywords: Duloxetine HCl (DUL), Methylcobalamin (MEC), Quality by Design (QbD), HPTLC, Validation

INTRODUCTION

Quality by design is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [1]. Traditional chromatographic method development has always involved the time-consuming process of varying one system parameter at a time, examining its effect on the method and system operation. This generally requires a large number of experimental runs and in most situations the developed method requires further development [2].

The objective of the QbD initiative is to demonstrate both understanding and control of pharmaceutical processes to deliver high-quality pharmaceutical products while affording opportunities for continuous improvement. QbD delivers a better understanding of method capabilities and limitations; ensures a superior chance of successful downstream method validation and transfer. The QbD concept can be extended to analytical methods [3]. The analytical methods used for the analysis of active pharmaceutical ingredients (API) and drug products are an integral part of the QbD.

Duloxetine HCl (DUL)-(3*S*)-*N*-methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine hydrochloride has an empirical formula of $C_{18}H_{19}NOS$. HCl and a molecular weight of 333.38 g/mol (fig. 1(A)) [4]. It is a potent inhibitor of serotonin and norepinephrine reuptake and thus it is used for major depressive disorders [5-7], anxiety disorder, and pain associated with diabetic peripheral neuropathy or fibromyalgia. Furthermore, it provides evidence of an effect on pain in the case of urinary incontinence [8, 9] independent of its effect on depression. Therefore, Duloxetine HCl is used in the treatment of the different symptoms of depression [10].

Methylcobalamin (fig. 1(B)) is MeCbl; Coα-[α-(5,6-dimethylbenz-1H-imidazolyl)]-Co β methylcobamide and has an empirical formula $C_{63}H_{91}CoN_{13}O_{14}P$. It is a cobalamin, and it is a form of Vitamin B_{12}. Vitamin B_{12} is used in the body in two forms such as Methylcobalamin and 5-deoxyadenosyl cobalamin.

The methionine synthase is an enzyme responsible for the conversion of the amino acid homocysteine into methionine and this enzyme requires Methylcobalamin as a cofactor. Methylcobalamin is also used in the treatment of peripheral neuropathy, diabetic neuropathy, hearing loss, Alzheimer's disease and as a preliminary treatment for amyotrophic lateral sclerosis [11].

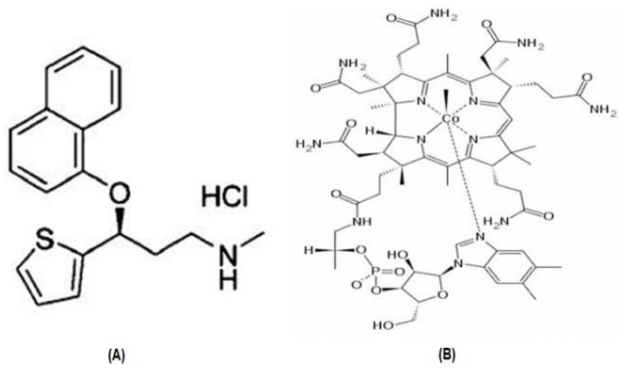

Fig. 1: Chemical structure of duloxetine hydrochloride (A) and methylcobalamin (B)

The combined dosage form of these drugs is used for the treatment of neuropathic pain associated with peripheral neuropathy especially diabetic neuropathy. Duloxetine HCl is not official in any pharmacopoeia. Methylcobalamin is official in Japanese Pharmacopoeia [12]. The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of Duloxetine HCl and Methylcobalamin in their combined dosage forms. A literature survey indicated few analytical methods like Spectrofluorimetric method [13], gas chromatography [14], MS, NMR spectrometry, and X-ray analysis [15, 16], HPLC and HPTLC methods [17-32], UV Spectrophotometric method [33-41] and capillary electrophoresis with laser-induced fluorescence detection method [42] which have been reported for the determination of DUL and MEC in individual or combination with other drugs in pharmaceutical dosage form and in biological samples. This paper aims to describe the development and validation of new, simple and robust HPTLC method for the simultaneous determination of Duloxetine HCl and Methylcobalamin in standard and in its pharmaceutical dosage form by implementing the Quality by Design approach.

MATERIALS AND METHODS

Reagents and materials

Duloxetine HCl (DUL) and Methylcobalamin (MEC) were procured as gratis samples from Sunrise Pharma Pvt Ltd (Satej, Ahmedabad, India). Capsule formulation, Duzela® M 30 (Sun Pharma laboratories ltd., India), was obtained commercially with the labelled amounts of 30 mg of DUL and 1.5 mg of MEC. Analytical reagents propanol and strong ammonia were purchased from Merck, Mumbai, India. HPLC grade methanol was purchased from Finar Chemicals Ltd., Ahmedabad, India. HPLC grade water was purchased from Rankem, Ankleshwar, India. Aluminium-backed silica gel 60 F-254 plates (#1.05554, 20 cm × 10 cm: 200 µm thickness) were purchased from E. Merck (Darmstadt, Germany).

Equipment and chromatographic conditions

Chromatographic separation of the drugs was performed by a CAMAG HPTLC system equipped with Linomat V autosampler, TLC scanner III with winCATS 1.2.2 software (CAMAG, Muttenz, Switzerland). The prepared dilution of standards was spotted in the form of bands of width 6 mm with a Hamilton 100 microliter syringe, Camag on pre-coated silica gel aluminium Plates 60F-254 (20 cm × 10 cm, 200 µm thickness, Merck, Darmstadt, Germany) using a Linomat V sample applicator. The plates were prewashed by methanol and activated at 60 °C for 2.5 min prior to chromatography. A constant application rate 150 nl/s was employed and the space between the two bands was 12 mm.

The slit dimension was kept at 5 mm × 0.45 mm and 20 mm/s scanning speed was employed. The mobile phase consisted of propanol: water: 25% v/v strong ammonia solution (9:2:1, v/v/v). Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber saturated with the mobile phase to a distance of 85 mm. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (25±2 °C) and at a relative humidity of 55±5 % RH. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning performed on Camag TLC scanner III in the absorbance mode was scanned in the range of 200-300 and select the optimised wavelength at 280 nm. The source of radiation utilized was a deuterium lamp. The evaluation was done using linear regression analysis via peak areas.

Method development

Based on sample solubility, pKa value, and solvent polarity, various mobile phase compositions were tried to get a good separation. The standard solution containing a mixture of DUL and MEC as well as individual drugs were run in different mobile phases in order to find the best conditions for separating both the drugs simultaneously. The composition of mobile phase for separation was determined to be propanol: water: 25% v/v ammonia solution. For quantitative analytical purposes, the wavelength was set at 280 nm as the optimum wavelength.

Solution preparation

Preparation of standard solution

A standard stock solution mixture was prepared in methanol containing 3000 µg/ml of DUL and 150 µg/ml of MEC. Pipette out 1 ml from standard stock solution into a 10 ml volumetric flask and makeup with methanol to get the working standard solution containing 300 ng/µl of DUL and 15 ng/µl of MEC.

Preparation of sample solution

Take twenty capsules, powdered the inner contents to fine powder (each capsule containing 30 mg of DUL and 1.5 mg of MEC). Weigh and transfer equivalent to 300 mg of DUL and 15 mg of MEC into a 100 ml volumetric flask. Add 60 ml methanol in a volumetric flask and sonicate for 30 min with intermediate shaking. Make up volume with methanol up to 100 ml and prepared stock solution containing 3000 µg/ml of DUL and 150 µg/ml of MEC. Pipette out 1 ml from sample stock solution into a 10 ml volumetric flask and makeup with methanol to get the working sample solution containing 300 ng/µl of DUL and 15 ng/µl of MEC.

Software aided method optimization

Response surface methodologies, such as the Box-Behnken and Central Composite Design (CCD), were the models possible curvature in the response. Central Composite Design, statistical screening design, was used to optimize the compositional parameters and to evaluate interaction effects and quadratic effects of the chromatographic conditions like the mobile phase composition and saturation time on retardation factor (R_f). A response surface design is suitable for exploring quadratic response surfaces and constructing polynomial models with Design Expert_ (Version 7.0.0.1, Stat-Ease Inc., Minneapolis, MN, USA) statistical software [43, 44]. The selection of critical factors and ranges examined for optimization was based on preliminary univariate studies of method development and chromatographic intuition. The Central Composite design matrix comprising of 13 experimental runs was constructed. The non-linear computer generated quadratic model is given as

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon$$

Where, Y is the measured response retardation factor (R_f) associated with each factor level combinations; mobile phase composition (water content) (X_1) and saturation time (X_2). The composition of the mobile phase refers to the volume of water with respect to the total volume of the mobile phase. The dependent and independent variables selected are shown in table 1 along with their low, medium (nominal value) and high levels, which were selected based on the results from preliminary experimentation. The nominal value for two factors, X_1 and X_2 were 2 ml and 20 min, respectively. Accordingly, the water content (X_1) was maintained between 1.5 ml and 2.5 ml. Similarly, the low and high values of the chamber saturation time (X_2) were fixed at 15 min and 25 min, respectively.

Table 1: Variables selected in central composite design

Factor-independent variables	Levels used		
	Low (-1)	Medium (0)	High (+1)
X_1 = Water content (ml)	1.5	2.0	2.5
X_2 = Saturation time (min)	15	20	25
Dependant variables	Constraints		
Y_1 (retardation factor of DUL)	$0.68 \leq Y_1 \geq 0.80$		
Y_2 (retardation factor of MEC)	$0.51 \leq Y_2 \geq 0.58$		

A total of thirteen experiments with five centre points were conducted by selecting two factors; the water content in the mobile phase (X_1), the chamber saturation time (X_2) and the R_f of DUL and MEC were the responses selected for both drugs depicted in table 2. Replicates (n = 5) of the centre points were performed to estimate the experimental error. The adequacy of the model was confirmed by considering R-square value of regression analysis, standard deviation (SD), predicted residual sum of square (PRESS) and coefficient of variance (% CV).

Statistical analysis of model by analysis of variance (ANOVA)

The significance of the relevant factors was calculated using Fisher's statistical test for the Analysis of Variance (ANOVA) model. The significance of the model was examined using a lack of fit test, p-value and the model F-value. ANOVA for non-linear regression was partitioned the total variation of a sample into components. These components were then used to compute an F-ratio that evaluated the effectiveness of the model. If the probability associated with the F-ratio is low, the model is considered a better statistical fit for the data. All experiments were conducted in a randomized order to minimize the bias effects of uncontrolled variables.

Calibration curve of duloxetine HCl and methylcobalamin

A working standard mixture solution was prepared in methanol containing 300 µg/ml of DUL and 15 µg/ml of MEC. Different volumes of working standard solution ranging from 4, 6, 8, 10 and 12 µl were spotted on the TLC plate (10 × 10 cm) to obtain concentrations of 1200, 1800, 2400, 3000 and 3600 ng/spot of DUL and 60, 90, 120, 150 and 180 ng/spot of MEC, respectively. Each concentration was spotted five times on the TLC plate. The data of peak areas plotted against the corresponding concentrations were treated by linear least-square regression analysis.

Table 2: Observed responses in central composite design for 13 analytical trials

Run	Independent variable		Dependent variable	
	Water content (X_1) (ml)	Saturation time (X_2) (min)	R_f (DUL) (Y_1)	R_f (MEC) (Y_2)
1	2.50	25	0.74	0.58
2	2.50	20	0.69	0.57
3	2.00	25	0.78	0.55
4	1.50	20	0.79	0.52
5	2.00	15	0.75	0.54
6	2.00	20	0.77	0.55
7	2.00	20	0.76	0.55
8	1.50	15	0.80	0.51
9	2.00	20	0.77	0.55
10	2.00	20	0.76	0.55
11	2.50	15	0.68	0.54
12	1.50	25	0.80	0.53
13	2.00	20	0.77	0.55

R_f = Retardation factor

Method validation

The analytical method was validated for precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness in accordance with ICH guidelines [45].

Precision

Intraday and Interday precision study of DUL and MEC was carried out by estimating corresponding responses 3 concentrations and 6 replicates on the same day and on 3 different days for the concentration covering the specified range of Duloxetine HCl (1500, 3000, 4500 ng/spot) and Methylcobalamin (75, 150, 225 ng/spot).

Accuracy, as recovery

Accuracy was evaluated in triplicate, at three different concentrations equivalent by spiking to 80, 100, and 120% of the active ingredient, by adding a known amount of DUL and MEC standard to a sample of known concentration and calculating the recovery of DUL and MEC, and % RSD for each concentration. The previously analyzed samples (3 µg for DUL and 0.15 µg for MEC) were spiked with extra concentration levels of 2.4, 3.0 and 3.6 µg for DUL and 1.2, 1.5 and 1.8 µg for MEC and the mixtures were reanalyzed by the developed Method. The recovery studies were carried out in triplicates each level.

Limits of detection (LOD) and limit of quantification (LOQ)

Limit of Detection (LOD) is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. The limit of quantitation (LOQ) is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ were calculated using the equations as per ICH guideline.

$$LOD = 3.3 \times (SD/S)$$

$$LOQ = 10 \times (SD/S)$$

Where, SD is standard deviation of the peak area (n=5), taken as measure of the noise and S is the slope of the corresponding calibration curve.

Robustness

The robustness of the method was investigated by making small deliberate changes in the chromatographic conditions at two different levels. The deliberate changes for selected chromatographic conditions were different compositions of the mobile phase e. g. propanol: water: 25% v/v ammonia solution (9.5:1.5:1.0, 9.0:2.0:1.0 and 8.5:2.5:1.0 v/v/v) and chamber saturation time (15, 20 and 25 min).

Analysis of the marketed formulation

Weigh and determine the average weight of twenty capsules contents (Duzela® M 30; label claim 30 mg DUL and 1.5 mg MEC) without shell. A portion of crushed powder contents equivalent to the weight of one capsule was accurately dissolved in methanol and sonicate for 20 min to achieve complete dissolution of DUL and MEC. Dilution was done to get a stock solution of DUL (300 ng/µl) and MEC (15 ng/µl). The resulting solution was filtered and applied 10 µl (3000 ng/spot of DUL and 150 ng/spot of MEC) on TLC plate followed by development and scanning. Peak area was measured, and % assay was calculated. The response was an average of six determinations.

RESULTS AND DISCUSSION

Selection of wavelength

The sensitivity of the HPTLC method with ultra-violet detection depends on the use of an appropriate wavelength. The developed plate was subjected to densitometric measurements in a scanning mode in the UV region of 200-400 nm and the overlain spectrum was recorded on a CAMAG TLC Scanner 4. Both drugs are appreciably absorbed light at 280 nm and this wavelength was selected as the detection wavelength (fig. 2).

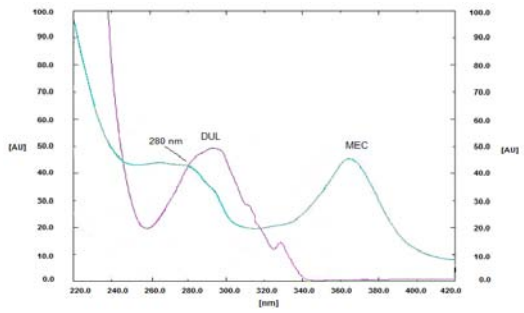

Fig. 2: Overlain UV spectra of duloxetine HCl (DUL) and methylcobalamin (MEC)

Method optimization

The chromatographic conditions were optimized in order to develop an HPTLC method for the simultaneous measurement of DUL and MEC in standard and pharmaceutical dosage form.

Preliminary study

Various combinations of solvents in different ratios like methanol, water, ethyl acetate, chloroform, toluene, ethanol, propanol, diethyl ether and 25% v/v strong ammonia solution were tried for resolving the peaks of DUL and MEC. Various combinations of propanol and water were tried in different ratios as good resolution between two drugs was observed. Propanol: water (9:2) gave good resolution, but typical peak shape of MEC was lost. Strong ammonia (25% v/v) was added to improve peak characteristics. So, the final optimized mobile phase, propanol: water: 25% v/v strong ammonia (9:2:1), gave highest resolution and R_f values of 0.77±0.01 and 0.55±0.02 for DUL and MEC, respectively (fig. 3) at a fixed chamber saturation time of 20 min. The R_f value of both drugs was also minutely affected by the chamber saturation time. Therefore, further chromatographic conditions were optimized to obtain well-defined, compact bands of DUL and MEC with acceptable R_f values between 0.2 and 0.8 for both drugs using CCD.

Optimization of chromatographic conditions using CCD

CCD was selected due to its flexibility and applied to optimize the HPTLC separation by gaining a better understanding of the factor's main and interaction effects. A Response surface-central composite statistical experimental design was employed using 13 experimental runs that included five centre points. The independent variables (such as the water content in the mobile phase (X_1) and chamber saturation time (X_2)) and the responses for all 13 optimized trial experimental runs are summarized in table 2.

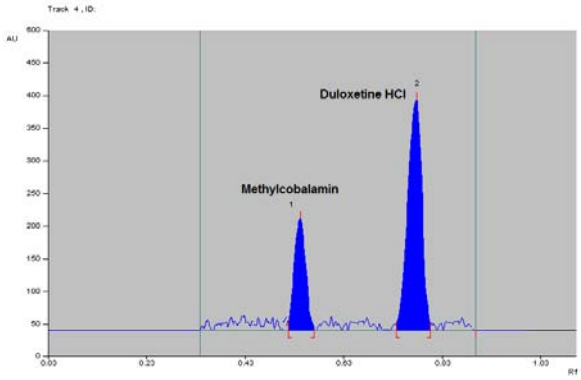

Fig. 3: Chromatogram of duloxetine HCl (Retardation factor (R_f): 0.77) and methylcobalamin (Retardation factor (R_f): 0.55) in Standard preparation

The result of regression analysis (table 3) having low standard deviation (SD) values 0.006 and 0.004, higher R-square values 0.9801 and 0.9596, lower predicted residual sum of square (PRESS) values 0.0017 and 0.0016 for Y_1 and Y_2, respectively indicated an excellent adequacy of the regression model. The higher value of correlation coefficient (R^2) signified an excellent correlation between the independent variables. It was observed that the best-fitted model was the quadratic model. Here, the adjusted R^2values were reasonable agreement with the predicted value (R^2≥0.88) which indicated the experimental data fitted regression equations well [46]. A coefficient of variation (% CV), a measurement of the reproducibility of the model, was less than 10%. An adequate precision, a measure of the signal (response) to noise ratio, greater than 4 is desirable. The obtained ratio for both drugs (26.445 for DUL and 20.111 for MEC) indicated an adequate signal. These models can be used to navigate design space. The final equation, in terms of the actual components and factors, is shown in table 3. A positive value represented an effect that favours optimization, whereas a negative value indicated an inverse relationship between the factor and the response.

Table 3: Summary results of regression analysis for quadratic model and responses *(Y)*

Response	Models	Adjusted R^2	Predicted R^2	SD	PRESS	%CV	Adequate precision
Retardation factor of DUL (Y_1)	Quadratic	0.9659	0.8963	0.006	0.0017	0.91	26.445
	Regression equation	$Y_1 = 0.76 - 0.047X_1 + 0.015X_2 + 0.015X_1X_2 - 0.018X_1^2 + 0.0067X_2^2$					
Retardation factor of MEC (Y_2)	Quadratic	0.9307	0.8884	0.004	0.0016	0.89	20.111
	Regression equation	$Y_1 = 0.55 + 0.022X_1 + 0.012X_2 + 0.005X_1X_2 - 0.005X_1^2 - 0.005X_2^2$					

R^2= Correlation coefficient; SD = Standard deviation; PRESS = Predicted residual sum of square; %CV = Coefficient of variance

The model was also validated with an analysis of variance (ANOVA) using the Design Expert software and the results are presented in table 4. The model was examined using a lack of fit test which indicated an insignificant lack of fit value corresponding with a higher p-value as compared to the model F-value. From the results of ANOVA (table 4), the Model F-value of 68.92 (DUL) and 33.23 (MEC) implied that the model was significant.

Table 4: Summary results of ANOVA statistical analysis for models and response (*Y*-retardation factor) for the finally suggested quadratic model

Source	Sum of Squares		Degree of freedom		Mean Square		F-Value		P*-Value	
	DUL	MEC	DUL	MEC	DUL	MEC	DUL	MEC	DUL	MEC
Model	0.016	0.0039	5	5	0.003248	0.000791	68.92	33.23	<0.0001	<0.0001
Water content	0.013	0.0028	1	1	0.013	0.0028	277.27	118.3	<0.0001	<0.0001
Saturation time	0.00135	0.00081	1	1	0.00135	0.00081	28.65	34.30	0.0011	0.0006
Residual	0.000329	0.00016	7	7	0.000047	0.000023	---	---	---	---
Lack of fit	0.000209	0.00016	3	3	0.000069	0.000055	2.33	---	0.2156	---

*P–Value (probability)<0.05 indicate model terms are significant.

The "Lack of fit F-value" of 2.33 implied the Lack of fit is not significant relative to the pure error. There was a 21.56% chance that a "Lack of fit F-value" this large could occur due to noise. Non-significant lack of fit is good to fulfill the fit model requirement. A model p-value of <0.0001 indicated that 0.01% chance for large "Model F-value" due to noise. P-values less than 0.05 indicated model terms were significant [47].

Three-dimensional response surface plots and perturbation plots were constructed to evaluate the effect of the factors on the retardation factor of each drug. Perturbation plots reveal the change in response from its nominal value for both drugs with all factors held constant at a reference point, and steepest slope or curvature indicates sensitiveness to a specific factor. The water content in the mobile phase had a most important effect on retardation factor, as increasing levels of water content resulted in a decrease in the retardation factor of DUL and increased in the retardation factor of MEC (fig. 4 (A) and (B)). However, the variation in retardation factor due to change in water content of mobile phase was acceptable as an evident of a robust method. It was found that a variation in the R_f value of DUL and MEC respectively as a function of the water content and the chamber saturation time (fig. 5(A) and (B)). The second factor, chamber saturation time, had no significant effect on retardation factor.

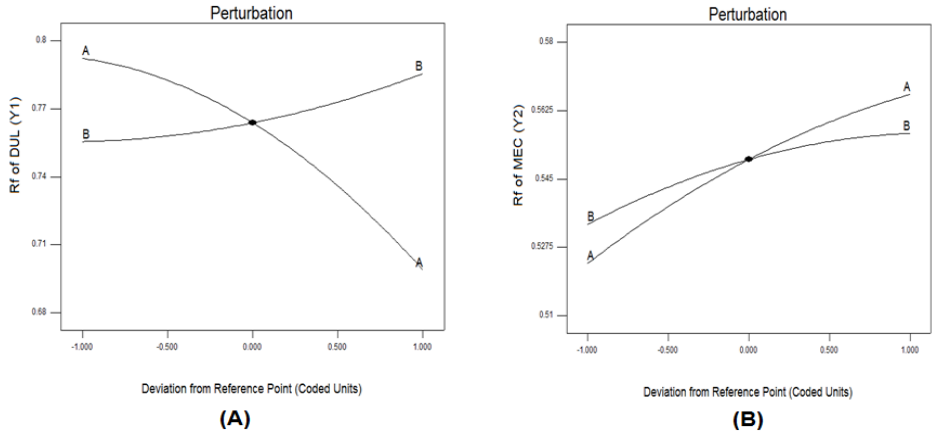

Fig. 4: Perturbation graph showing the effect of factor, A (water content) and B (chamber saturation time), on the Retardation factor (R_f) of Duloxetine HCl (A) and Retardation factor (R_f) of Methylcobalamin (B)

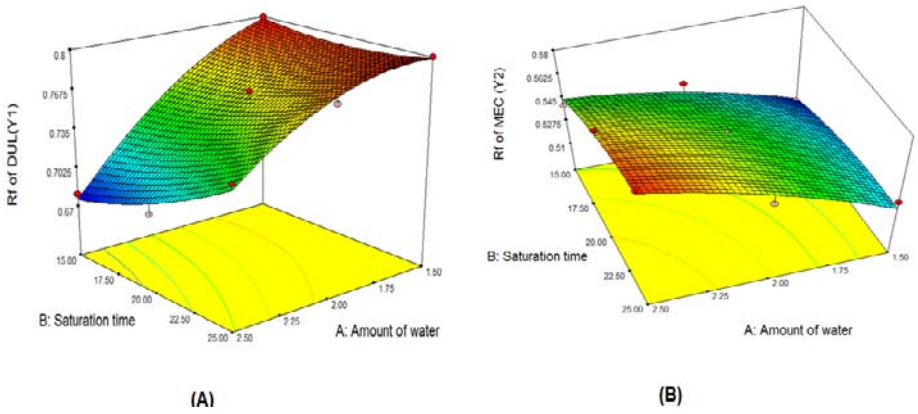

Fig. 5: 3D Response surface plot showing the effect of mobile phase composition (water content) and saturation time on retardation factor (R_f) of Duloxetine HCl (A) and retardation factor (R_f) of Methylcobalamin (B)

In order to get the best chromatographic performance, the multicriteria methodology was employed by means of Derringer's desirability function. Individual desirability functions range from 0 (undesired response) to 1 (fully desired response). A value of D close to 1 indicates that combination of different criteria is globally optimal. The red area in desirability plot indicates prediction at all points in this region is one. The yellow area in overlay plot indicates all the constraints are satisfied in this region. Desirability and overlay plot were obtained from the model for the selected responses (fig. 6 and 7). The agreement between the experimental and predicted responses for the predicted optimums, 1 (X_1=1.94 ml and X_2=23.44 min) and 2 (X_1=2.37 ml and X_2=16.55 min), were selected for investigating the predictability of the proposed model. In the case of optimum 1, the R_f for DUL was obtained 0.77 for experimental and 0.78 for predicted values with % percentage error 0.09 whereas the R_f for MEC was obtained 0.54 and 0.55 for experimental and predicted values, respectively with % percentage error 0.51. In the case of optimum 2, the R_f for DUL was obtained 0.69 for experimental and 0.70 for predicted values with % percentage error 2.01 whereas the R_f for MEC was obtained 0.54 and 0.55 for experimental and predicted values, respectively with % percentage error 1.90. The percentage of the prediction error was calculated using the following formula: predicted error = experimental-predicted/predicted × 100. The above results and % predicted error identified a set of coordinates that produced a high desirability value (D = 1) at optimum condition 1. Thus, these coordinates were used to select an optimum experimental condition to analyze DUL and MEC in combination. The selected optimized condition was propanol: water: ammonia solution (25% v/v) (9:2:1 v/v/v) as solvent system and 20 min as saturation time showed good agreement, resolution with peak area and R_f values of both drugs (0.77±0.02 for DUL; 0.55±0.03 for MEC).

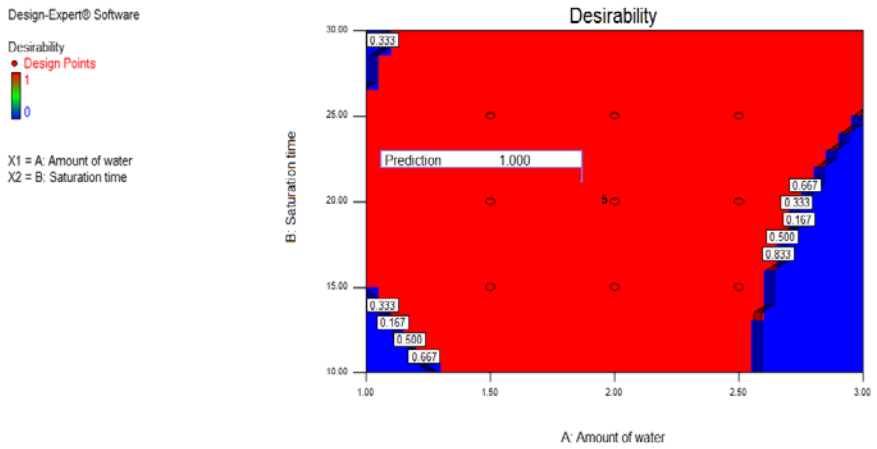

Fig. 6: Desirability showing the effect of mobile phase composition (water content) and saturation time on retardation factor of Duloxetine HCl and Methylcobalamin

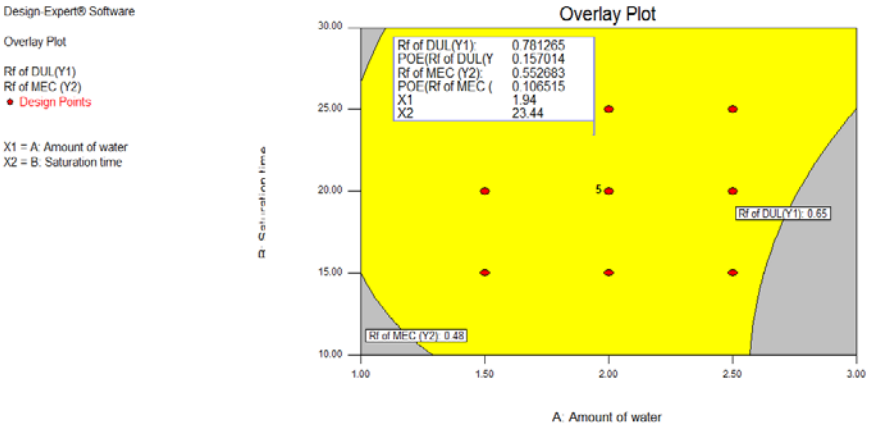

Fig. 7: Overlay plot showing the effect of mobile phase composition (water content) and saturation time on retardation factor of Duloxetine HCl and Methylcobalamin

Table 5: Linear regression data for calibration curve (n=5)

Parameters	DUL	MEC
Linearity range (ng/spot)	1200-3600 ng/spot	60-180 ng/spot
Regression equation	$Y = 6.013x + 316.4$	$Y = 7.553x - 1.2$
Correlation coefficient	0.999	0.998
Slope[a)]±SD	6.013±0.005	7.553±0.020
Intercept[a)]±SD	316.40±2.40	-1.20±2.62
LOD	113.39	6.68
LOQ	343.62	20.24

[a)]n=5 replicate analysis for each concentration of linearity range; SD = standard deviation

Calibration curves

The linear regression data for the calibration curves (n=5) as shown in table 5 showed a good linear relationship over the concentration range of 1200-3600 ng/spot for DUL and 60-180 ng/spot for MEC with respect to peak areas. No significant difference was observed in the slopes of standard curves. 3D Calibration linearity graph of DUL and MEC at 280 nm was obtained (fig. 8).

Validation of the method

Precision

The repeatability of sample application and measurement of peak areas were expressed in terms of relative standard deviation (% RSD). The results depicted in table 6 revealed intra-day and inter-day variation covering the specified range of Duloxetine HCl (1500, 3000, 4500 ng/spot) and Methylcobalamin (75, 150, 225 ng/spot). The low value of the RSD was indicative of the repeatability of the method.

Limit of detection (LOD) and quantification (LOQ)

LOD and LOQ were estimated using standard deviation (SD) of the lowest response and slope of the calibration curve. LOD and LOQ were found to be 113.39 ng and 343.62 ng for DUL and 6.68 ng and 20.24 ng for MEC, respectively.

Recovery studies

By spiking previously analyzed test solution 3 µg (DUL) and 0.15 µg (MEC) with the additional standard drug, the recovery of the method was found to be varied from 98.82 to 100.12% for DUL and from 98.97 to 100.63% for MEC. Values of % recovery and % RSD were listed in table 7. The value of % RSD<2% indicated that the method was accurate.

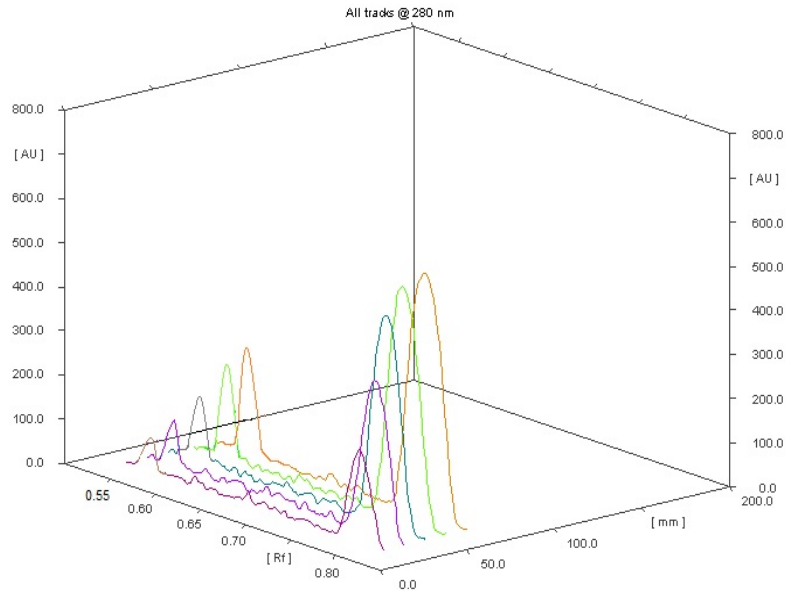

Fig. 8: 3D Calibration linearity graph of Duloxetine HCl (1200-3600 ng/spot, R_f at 0.77±0.01); and Methylcobalamin (60-180 ng/spot, R_f at 0.55±0.02) at 280 nm

Table 6: Intra-day and Inter-day precision (n=6)

Drug	Amount (ng/spot)	Intra-day precision		Inter-day precision	
		Mean %assay[b)]±SD	%RSD	Mean %assay[b)]±SD	%RSD
DUL	1500	99.57±0.95	0.95	99.30±1.17	1.18
	3000	99.20±0.72	0.73	99.23±0.72	0.73
	4500	99.35±0.71	0.71	99.18±0.58	0.58
MEC	75	98.70±0.54	0.54	98.93±0.83	0.84
	150	98.77±0.55	0.56	99.56±0.56	0.56
	225	99.88±1.17	1.17	98.94±1.17	1.18

[b)] n=6 replicate analysis for each interval; SD = standard deviation; RSD = relative standard deviation

Table 7: Results of recovery studies by standard addition method

	Amount taken	Amount added	Total amount recovered [(c)] (mean±SD)	Mean % recovery	% RSD
For DUL	3000 ng	2400 ng	5460.09±48.99	101.11	0.90
	3000 ng	3000 ng	6009.49±34.08	100.16	0.57
	3000 ng	3600 ng	6519.56±26.44	98.78	0.41
For MEC	150 ng	120 ng	272.15±3.31	100.80	1.22
	150 ng	150 ng	299.29±2.73	99.76	0.91
	150 ng	180 ng	331.40±2.29	100.42	0.69

[c)] Three independent analyses at each level; SD = standard deviation; RSD = relative standard deviation

Table 8: Results from robustness experiments

Condition	Value	Mean % recovery		% RSD		Retardation factor (R_f)[(d)]±SD	
		DUL	MEC	DUL	MEC	DUL	MEC
Mobile phase ratio	Propanol: water: ammonia (8.5:2.5:1.0, v/v/v)	98.48	98.66	0.25	0.66	0.74±0.007	0.52±0.008
	Propanol: water: ammonia (9.5:1.5:1.0, v/v/v)	100.16	100.66	0.23	0.35	0.78±0.008	0.57±0.004
Saturation time	15 min	99.75	99.52	0.47	0.55	0.73±0.008	0.52±0.007
	25 min	99.93	98.95	0.19	0.44	0.79±0.010	0.59±0.007

[d)]Average of three measurements at each condition; SD = standard deviation; RSD = relative standard deviation

Robustness of the method

By introducing small changes in the mobile phase composition and chamber saturation time in chromatographic conditions and the results were examined. There were very slight changes in the peak area and retardation factor. The lower value of SD and % RSD indicated the robustness of method (table 8).

Analysis of duloxetine HCl and methylcobalamin formulation (capsule Duzela® M 30)

The validated method was used to estimate the DUL and MEC content of a commercially available brand of the capsule containing 30 mg of DUL and 1.5 mg of MEC. Recovery of DUL and MEC from capsule formulation were 99.28% (RSD 0.73%) and 98.77% (RSD

0.56%), respectively (table 9). The amounts measured were in good agreement with the label claims. The results of the assay indicated that the method was selective for analysis of DUL and MEC without interference from the excipients present (fig. 9).

Table 9: Results obtained by the proposed method for the assay of drugs in pharmaceutical preparations (Duzela® M 30 capsules (label claim 30 mg DUL and 1.5 mg MEC); (n = 6))

Parameter	DUL	MEC
Mean Peak Area	18449.33	1120.00
Recovery (%)	99.28	98.77
RSD (%)	0.73	0.56

RSD = relative standard deviation

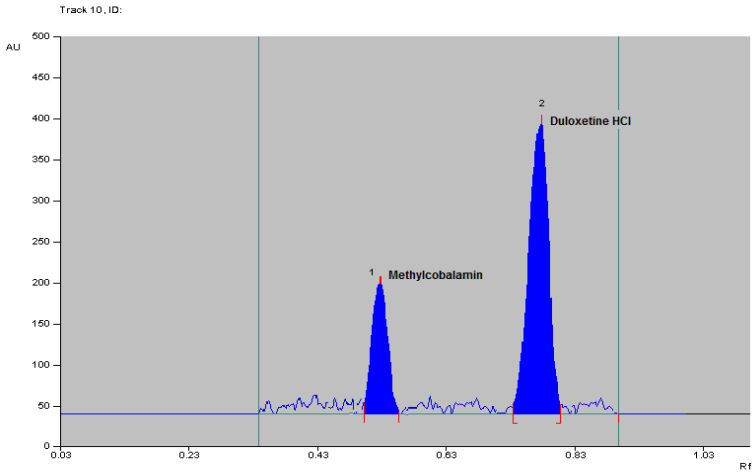

Fig. 9: Chromatogram of duloxetine HCl (Retardation factor (R_f): 0.77) and Methylcobalamin (Retardation factor (R_f): 0.54) in marketed formulation

CONCLUSION

A simple, rapid, accurate, precise, specific and reproducible HPTLC analytical method with UV detection was developed for the determination of Duloxetine HCl and Methylcobalamin in active pharmaceutical ingredients and in marketed capsule dosage form. A Quality by Design (CCD design and response surface methodology) approach was successfully applied for optimization of HPTLC method for estimation of Duloxetine HCl and Methylcobalamin. First, the method goals were clarified based on process understanding. The experimental design described the scrutinizing of the key components including water content and saturation time. Their interrelationship was studied, and optimized condition was obtained. A better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the method to meet their intended purpose was done. It gave good resolution between two peaks and reasonable retardation factors for both peaks. The obtained results indicate that the use of a CCD design and multi-criteria decision-making approach is a flexible procedure that can reduce the number of necessary experiments for the development and optimization of an HPTLC method. Furthermore, it is an economical method that can be used to generate a maximum amount of information in less time with a small number of experiments. The method was validated in accordance with ICH guidelines. The method seems to be suitable for the quality control in the pharmaceutical industry because of its high simplicity and reproducibility.

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CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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