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ARTICLE

Development and Validation of Stability Indicating Method for Simultaneous Estimation of Esomeprazole and Levosulpiride by HPTLC using Experimental Design

M.M. Pandey[1,2,✉], K.B. Prajapati[1], A.J. Vyas[1], A.M. Patel[1], N.K. Patel[1], A.I. Patel[1], A.B. Patel[1] and A. Chudasama[1]

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ABSTRACT

The present study examines simultaneous multiple response optimization using desirability function for the development of an HPTLC method to detect esomeprazole magnesium trihydrate and levosulpiride in pharmaceutical dosage form. HPTLC separation was performed on aluminium plates pre-coated with silica gel 60 F_{254} as the stationary phase using ethyl acetate:methanol:toluene:ammonia (7:1.5:1.5:0.1% v/v/v) as the mobile phase. Full factorial design applied for the optimization of degradation condition. Esomeprazole magnesium trihydrate and levosulpiride were subjected to acid, alkali hydrolysis, oxidation and photodegradation. Experimental full factorial design has been used during forced degradation to determine significant factors responsible for degradation and to optimize degradation conditions reaching maximum degradation. 3^2 and 2^3 full factorial design has been used for optimization of chromatographic condition in acid and base degradation study, respectively. Quantification was achieved based on a densitometric analysis of esomeprazole magnesium trihydrate and levosulpiride over the concentration range of 800-4000 ng/band and 1500-7500 ng/band, respectively at 254 nm. The method yielded compact and well-resolved bands at R_f of 0.70 ± 0.02 and 0.32 ± 0.02 for esomeprazole magnesium trihydrate and levosulpiride, respectively. The linear regression analysis for the calibration plots produced $r^2 = 0.9967$ and $r^2 = 0.9981$ for esomeprazole magnesium trihydrate and levosulpiride, respectively. Method is validated as per ICH (Q2)R1 guideline.

KEYWORDS

Densitometric analysis, Forced degradation, Esomeprazole magnesium trihydrate, Levosulpiride.

Author affiliations:

[1]Department of Pharmaceutical Quality Assurance, B.K. Mody Government Pharmacy College, Polytechnic Campus, Near Aji Dam, Rajkot-360003, India
[2]Amneal Pharmaceutical Pvt. Ltd., Murray Road, Hanover, New Jersey 07936, USA

✉To whom correspondence to be addressed:

E-mail: meenakshipandey1012@gmail.com

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INTRODUCTION

The purpose of stability testing is to provide evidence regarding the quality of a drug substance or drug product and how each may vary with time under the influence of a variety of environmental factors like temperature, humidity, light, *etc*. concept of stability testing without using of experimental design, it does not provide detailed information about stability conditions [1]. To apply experimental design to conduct stability testing are described general way and the exact stress conditions.

Experimental design can be used to revealed degradation conditions and variables which are most likely exert influence

in degradation study. Factorial design also addresses interaction between variables *viz.*, heat/pH, heat/time, *etc*., which may shows susceptibility of the drug to degradation. It provides ultimate choice of storage conditions. The present work directed toward the use of factorial design to bring forced degradation under various degradation conditions [2-4].

Esomeprazole magnesium trihydrate, chemically (*S*)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl sulfinyl]-3*H*-benzoimidazole magnesium trihydrate (Fig. 1a), is class of proton pump inhibitor that inhibits gastric acid secretion through inhibition of K^+/H^+ ATPase in gastric parietal cells [5]. Levosulpiride, *N*-[[(2*S*)-1-ethylpyrrolidin-2-yl]methyl]-2-methoxy-5-sulfamoylbenzamide (Fig 1b). It consists of blocking the D2 dopaminergic receptors, preferentially located on the presynaptic membranes in the dopaminergic pathways of the brain; this means that sulpiride is a selective auto-receptor blocker [6].

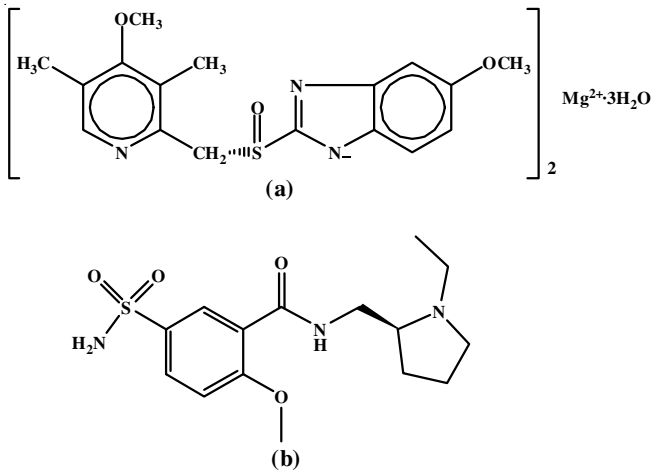

Fig. 1. Structure of drug (a) esomeprazole (b) levosulpiride

Literature survey reveals that various methods like HPLC, UPLC, UV spectroscopy and simple HPTLC were reported for determination of both title drugs alone and in combination. HPLC, UPLC methods are sophisticated, expensive as compared to HPTLC method [7-11]. Nowadays, HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimal sample preparation. Moreover, small amount of mobile phase is required thus reducing analysis time and cost. Till the date, stability indicating using experimental design does not performed on these two drugs. Thus, a simple and rapid stability indicating high performance thin layer chromatographic method has developed and validated for esomeprazole magnesium trihydrate and levosulpiride.

EXPERIMENTAL

Analytical pure samples of ESMO and LEVO were procured from Baroque Pharmaceutical Pvt. Limited Khambhat and Atur Instru chem, Baroda, respectively. The pharmaceutical dosage form used in this study was SOMPRAZ L was procured from local market (Apollo pharmacy, Baroda). The solvents and chemicals (AR grade) used in the study were procured from Merck, Mumbai.

Instrumentation: Micro syringe (Linomat syringe 659.0014, Hamilton-Bonaduz Schweiz), precoated silica gel 60F254 aluminium plates (20-10 cm, 250 µm thickness; Merck,), Linomat 5 applicator, twin through chamber, UV chamber, TLC scanner, Win CATS version 1.4.6 software were used of (Camag) and stat-ease design expert version 7.0.0 were used in the study. All drugs and chemicals were weighed on an electronic balance (AUW 220, Shimadzu).

Preparation of working standards: Accurately weighed each 10.0 mg of ESMO and 20 mg of LEVO in each 10 mL volumetric flask, add 5.0 mL of methanol sonicate both drug for 10 min and dilute up to the mark with methanol, to achieve a concentration of 1000 µg/mL and 2000 µg/mL.

Preparation of sample solutions: Twenty capsules (SOMPRAZ L, contain 40 mg of ESMO and 75 mg of LEVO per capsule) were weighed. Accurately weighed powder equivalent to 40 mg of ESMO and transferred into a 100 mL volumetric flask, add 50 mL methanol followed by sonication for 30 min and dilute up to 100 mL with methanol. Resulting solution was filtered through Wattman filter paper.

Chromatographic condition: Standard and sample solutions were spotted with a micro-syringe in the form of bands having a band width of 6 mm on a pre-coated silica gel aluminium Plate 60 F254 using a Camag Linomat 5 sample applicator. Linear ascending development was carried out in a twin trough glass chamber. Mobile phase consisted of ethyl acetate:methanol:toluene:ammonia (7.5:1.5:1.5:0.1, %v/v/v/v). Optimized the chamber for saturation for 10-12 min at room temperature (25 ± 2 °C) before chromatographic development. The length of the chromatographic run was 8.5 cm. Subsequent to the development, HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed using a Camag TLC scanner 4 with the win CATS software. All measurements were made in the reflectance absorbance mode at 254 nm, because both drugs shows appreciable absorbance at 254 nm. with a slit dimension of 6.00 mm × 0.30 mm (micro), scanning speed of 20 mm/s, data resolution of 100 m/step. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. The concentrations of both drugs and marketed formulation were determined the intensities of diffusely reflected lights and the data were evaluated using an ordinary linear regression analysis of peak areas.

Forced degradation study

For acid induced degradation: 10.0 mg of ESMO and 20.0 mg LEVO were transferred into 10 mL volumetric flask add 5 mL of methanol sonicate it for 5 min dilute up to the mark with methanol. Then 8.0 mL of this solution was withdrawn. Add 1 mL of HCl (0.01 M, 0.015 M, 0.020 M, 0.025 M, 0.05 M, 0.1 M, 1 M individually), placed at room temperature for 1 h, reflux 50 °C, 60 °C for 15 min and 30 min and neutralize with adding 1 mL of NaOH. Filter the above solution using Whatman paper and apply on HPTLC plates and analyse under optimized chromatographic conditions.

For base induced degradation: 10 mg of ESMO and 20 mg of LEVO were transferred into 10 mL volumetric flask and dissolved with 2 mL of methanol diluted up to mark with NaOH (0.1 M, 0.5 M, 1 M separately). The solutions were

refluxed at 60 °C for 30 min. 1 mL of solution was pipette out and add HCl solution for neutralization. Filter the above solution using Whatman paper and analyse under optimized chromatographic conditions.

Oxidative degradation: 10 mg of ESMO and 20 mg of LEVO transferred to 10 mL volumetric flask and diluted up to mark with hydrogen peroxide (3 % H_2O_2) and placed at room temperature for 1 h and analyses under optimized chromatographic conditions.

Photo degradation: The ESMO and LEVO standard powder (10 mg and 20 mg) were exposed to UV light (in a UV chamber) and sunlight for 5 h. Appropriate dilutions were made in methanol to obtain final concentration of 1000 µg/mL and 2000 µg/mL and analyses under optimized chromatographic conditions.

Software aided method optimization: A full factorial design (FFD) was used to optimize acid induced and base induced degradation study. Based on preliminary variable study critical factor were examined for method development and degradation study. The selection of critical factors and ranges examined for optimization was based on preliminary univariate studies of method development and degradation condition. Full factorial design (3^2 and 2^3) with 9 and 8 run included 2 variables study at 3 levels and 3 variables study at 2 levels of acid induced and base induced degradation study, respectively. Total nine experiment conducted of acid induced degradation study by selecting two factor molarity (A) and time (B) and 8 experiment conducted for base induced degradation study by selecting three factor temperature (A), time (B) and normality (C). The % degradation of both drugs were depicted in Tables 1 and 2. Minimum level of all two factors for acid induced degradation study 0.010 N (A) and 15 min (B) and for base induced degradation study 60 °C (A), 30 min (B) and 0.5N (C), respectively. Maximum level of all two factors for acid induced degradation study 0.020 N (A) and 45 min (B) and for base induced degradation study 80 °C (A), 60 min (B) and 0.1 N(C) respectively. A middle level for acid induced degradation study 0.015 N (A) and 30 min (B), respectively. Data generated were analyzed using the trial version of the State Ease Design Expert (Version 7.0.0) statistical software. The significance of the relevant factors were calculated using Fisher's statistical test for the analysis of Variance (ANOVA) model. All experiments were conducted in a randomized order to minimize the bias effects of uncontrolled variables.

TABLE-1
DESIGN DOMAIN OF FACTORIAL DESIGN WITH RESPONSE

Runs	Independent variables		Dependent variables	
	A: Molarity (M)	B: Time (min)	% Degradation of ESMO	% Degradation of LEVO
1	0.015	15	17.26	6.64
2	0.015	30	22.19	6.69
3	0.010	15	4.27	1.65
4	0.020	15	23.33	14.94
5	0.015	45	21.91	20.05
6	0.020	30	17.9	11.54
7	0.020	45	24.11	34.36
8	0.010	45	12.67	16.79
9	0.010	30	6.73	10.79

TABLE-2
DESIGN DOMAIN OF FACTORIAL DESIGN WITH RESPONSE

Runs	Independent variables			Dependent variable	
	A: Temp. (°C)	B: Time (min)	C: Normality (N)	% Degradation of ESMO	% Degradation of LEVO
1	60	30	1	5.31	57.68
2	60	60	1	27.17	73.02
3	80	60	1	37.31	41.46
4	60	60	0.5	13.13	54.79
5	80	30	0.5	16.69	67.83
6	60	30	0.5	4.27	42.46
7	80	30	1	23.97	25.78
8	80	60	0.5	18.4	77.95

Method validation

Linearity: Different volumes (1-7.5 µL) of standard solutions of both drugs were applied to the HPTLC plate to obtained concentration range of 800-4000 ng/band of ESMO and 1500-7500 ng/band for LEVO. Homoscedasticity of the variances along the regression line of each drug was verified using Bartlett's test.

LOD/LOQ: The limit of detection and limit of quantitation was calculated based on standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = 3.3σ/s and LOQ = 10σ/S as defined by ICH guidelines [12].

Precision: Precision of the developed method was evaluated by performing intra-day and inter-day precision studies. Intra-day precision was assessed based on three replicates of three different concentration (800, 2400 and 4000 ng/band for ESMO; 1500,4500 and 7500 ng/band for LEVO).

Accuracy: Accuracy was ascertained by performing recovery at three levels (50 %, 100 % and 150 %). Recovery studies were carried out by spiking three different amount of ESMO standard (800 ng, 1600 ng and 2400 ng) to the dosage form (40 ng/band). Similarly, spiking for LEVO standard was (1500 ng, 3000 ng and 4500 ng) to the dosage form (75 ng/band) by standard addition method.

Specificity: Specificity of the method was ascertained by comparing peak purity of standard drug with formulation and degradation sample. Spot for ESMO and LEVO in sample and degradation studies were confirmed by comparing the R_f values and spectra of the sample spot with that of standard. Peak purity of ESMO and LEVO were assessed by comparing the spectra at three different levels, *i.e.*, peak start(S), peak apex (M) and peak end (E) of the spot.

Robustness: Effect of small and deliberate variations in the method parameters, such as a change in the ethyl acetate and toluene content in the mobile phase by volume, saturation time, distance travelled or wavelength were evaluated.

Marketed formulation analysis: To determine the concentration of ESMO and LEVO in capsule dosage form (label claim: 40 mg and 75 mg per capsule), contents of 20 capsule were accurately weighed and finely powdered in glass mortar. An accurately weighed powder sample equivalent to 40 mg of ESMO was weighed and transferred into 100 mL volumetric flask containing 50 mL methanol, followed by sonication for 30 min and further dilution up to 100 mL with methanol. And the solution was applied on the TLC plate.

RESULTS AND DISCUSSION

Selection of wavelength: Developed plate was subjected to densitometric measurements in scanning mode in the UV-vis region of 200-700 nm. Both drugs appreciably absorbed light at 254 nm.

Method optimization: Chromatographic conditions were optimized in order to develop HPTLC method for the simultaneous measurement of ESMO and LEVO in standard and pharmaceutical dosage form. The optimized HPTLC chromatogram of levosulpiride and esomeprazole is shown in Fig. 2.

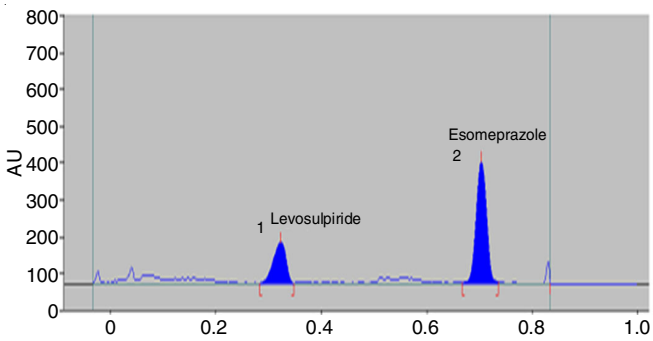

Fig. 2. Optimized HPTLC chromatogram of levosulpiride and esomeprazole

Optimization of degradation condition using FFD: FFD was selected to optimize the HPTLC separation by applying factor's main and interaction effects. Three levels and two level full factorial designs were employed using nine and eight experimental run. Independent variables, for three level FFD Molarity of HCl (A), time (B) and for two level FFD temperature (A), time (B) and normality (C) and dependent variable for all 9 and 8 optimized trial experimental runs are summarized in Tables 1 and 2, respectively.

Model was also validated with an analysis of variance (ANOVA) using the Design Expert software and results are presented in Tables 3 and 4. Significant effects had a *p* value less than 0.05. An adequate precision, a measure of the signal (response) to noise ratio was, greater than 4 which desirable and obtained ratio for both drugs indicated an adequate signal. Coefficient of variation (% CV), which measures reproducibility of the model, was less than 10 % and adjusted R^2 values were high, indicating a good relationship between experimental data and those of fitted models. Here, the adjusted R^2values were well within the acceptable limit of $R^2 \geq 0.80$, which indicated that the experimental data fitted polynomial equations well. Final equation, in terms of the actual components and factors, is shown in Tables 3 and 4. Positive value represents an effect that favours optimization, whereas a negative value indicates an inverse relationship between the factor and the response.

Three-dimensional response surface plots and perturbation plots were constructed to evaluate effect of the factors on the %degradation of each drug. In Fig. 3, perturbation plots were presented for predicted model to better understand

TABLE-3
PREDICTED RESPONSE MODELS AND STATISTICAL PARAMETERS OBTAINED FROM THE ANOVA FOR THREE LEVELS FFD

Response (% degradation)	Type of model	Polynomial equation model for Y	Adjusted R^2	Model P value	% CV	Adequate precision
ESMO	Quadratic	R1 = –2.10704 + 350.56409A + 0.020264B – 1.52689AB8556.64272A^2 + 1.53295E – 004B^2	0.9191	0.0175	6.51	12.358
LEVO	Linear	R2 = –18.07833 + 1053.66667A + 0.53300B	0.6637	0.0160	40.54	8.263

TABLE-4
PREDICTED RESPONSE MODELS AND STATISTICAL PARAMETERS OBTAINED FROM THE ANOVA FOR TWO LEVELS FFD

Response (% degradation)	Type of model	Polynomial equation model for Y	Adjusted R^2	Model P value	% CV	Adequate precision
ESMO	Linear	R1 = –39.29875 + 0.75213A + 0.67975B – 55.19500C – 0.013058AB + 0.55550AC + 0.82100BC	0.9981	0.0308	2.65	73.678
LEVO	Linear	R2 = –224.19875 + 4.08312A + 0.34042B + 356.56500C – 1.55833E – 00AB – 5.59950AC + 0.28567BC	0.9974	0.0359	1.64	61.862

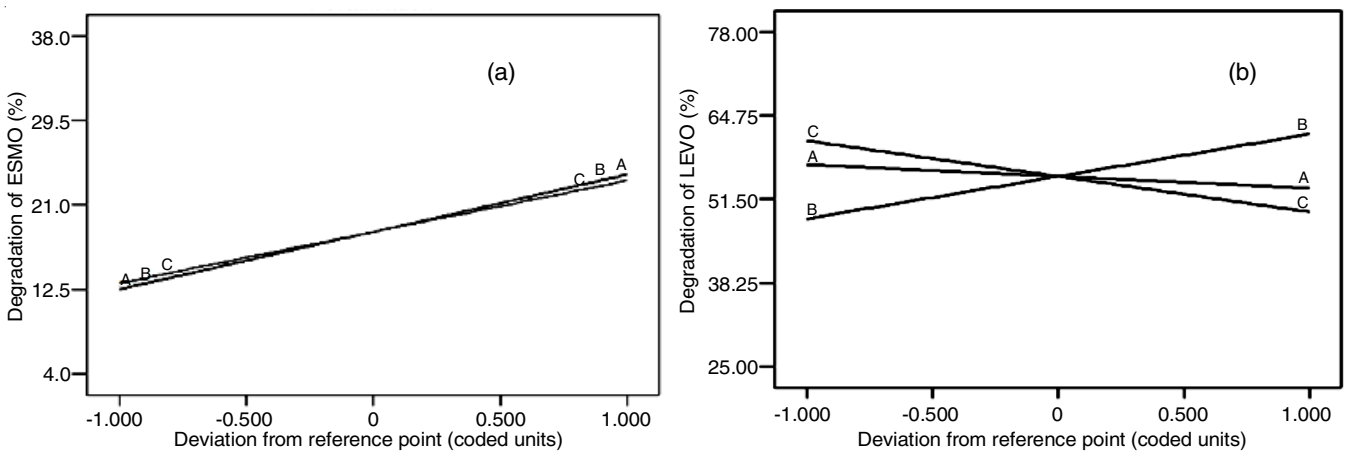

Fig. 3. Perturbation graph showing the effect of each factor, A, B and C on the (a) %degradation of ESMO and (b) %degradation of LEVO

investigated procedure. Fig. 3a, it can be concluded that the factor A, molarity has most prominent effect on the response R1 because of the sharp curvature in plot compare to factor B, Time. From Fig. 3b, it can be concluded that the factor B time has most prominent effect on the response R1 because of a steepest slope in compare to factor A, molarity. Fig. 3a, it can be concluded that the factor A, B and C has most prominent effect on the response R1 because of the steepest slope of all three factor but factor C gave maximum effect on factor B and factor A. From Fig. 3b, it can be concluded that factor B time has most prominent effect on the response R1 because of a steepest slope in compare to factor A and factor B.

Optimum conditions of separation were estimated using Derringer's desirability function. During the numerical optimization, the targets of individual factors and responses were fixed (Fig. 4). For the different solutions of the optimization provided by the software, two conditions that have a desirability near 1 were selected.

To investigate predictability of the proposed model, agreement between experimental and predicted responses for the predicted optimums, 1 for acid and base induced degradation study are shown in Table-5. Percentage of the prediction error was calculated using the following formula:

$$\text{Predicted error } (\%) = \frac{\text{Experimental} - \text{Predicted}}{\text{Predicted}} \times 100$$

Table-5 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 degradation condition to analyze ESMO and LEVO in acid and base induced degradation. Selected optimized composition for the final HPTLC analysis was 0.020M HCl at 15 min and 0.5N HCl 70 ºC at 30 min for acid and base, respectively. Under optimized degradation condition % degradation of ESMO and LEVO 23.0125 and 10.99 for acid induced degradation, respectively. Same as % degradation of ESMO and LEVO 11.84 and 57.80 for base induced degradation, respectively (Fig. 5).

TABLE-5
COMPARISON OF EXPERIMENTAL AND PREDICTED VALUES OF DIFFERENT EXPERIMENTAL RUNS UNDER OPTIMUM CONDITIONS

Optimum condition	Value	% Degradation of ESMO	% Degradation of LEVO
1	Experimental	21.82	7.07
	Predictive	23.0125	10.99
	Predicted error	5.18	35.66
Optimum condition	Value	% Degradation of ESMO	% Degradation of LEVO
1	Experimental	17.84	63.35
	Predictive	11.84	57.80
	Predicted error	50.67	9.60

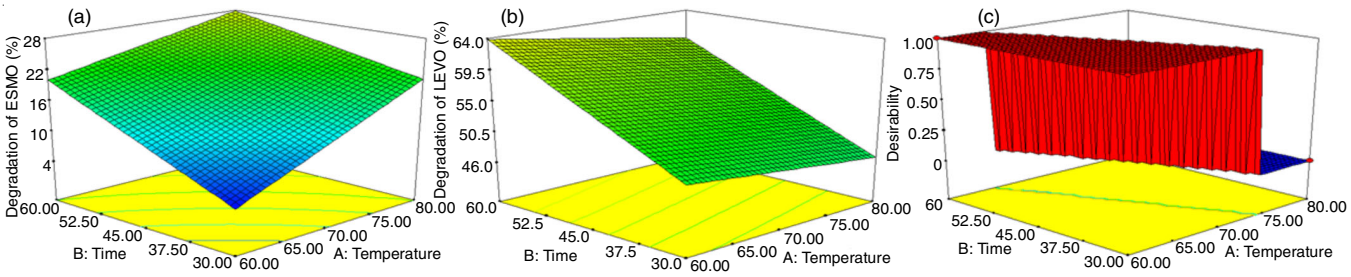

Fig. 4. Three-dimensional plots of the RSM for both responses (a) variation in the % Degradation of ESMO as a function of factor A & B for fixed value of C; (b) variation in the % Degradation of LEVO as a function of factor A & B for fixed value of C; (c) graphical representation of the maximum of Derringer's desirability function

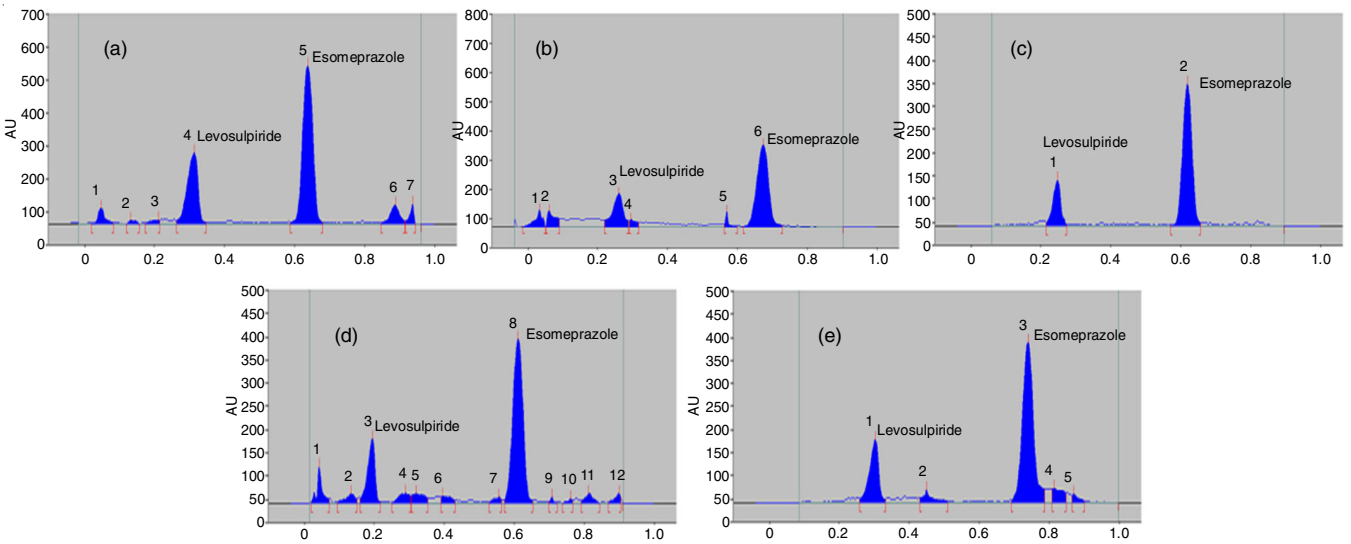

Fig. 5. Optimized HPTLC chromatogram for determination of stability of ESMO & LEVO drugs. (a) 0.020 M HCl at 15 min (b) 0.5 M NaOH 70 °C at 30 min (c) 3 %H_2O_2 R.T 1 h, (d) Sunlight 1 h, (e) UV chamber 5 h

Method validation

Linearity: The linearity of ESMO and LEVO showed correlation coefficient (r^2 = 0.9967 for ESMO and r^2 = 0.9981 for LEVO) in proposed concentration ranges of 800-4000 ng/band for ESMO and 1500-4500 ng/band for LEVO and caloberation band is shown in Fig. 6.

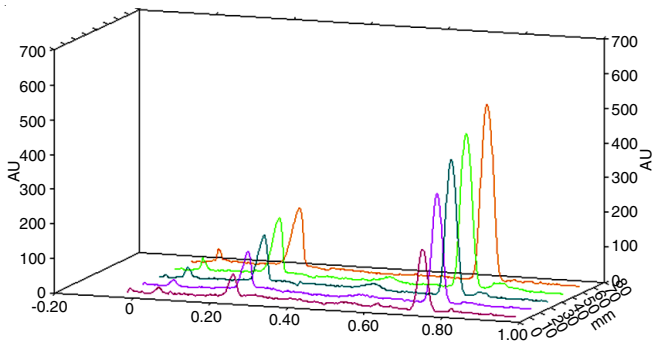

Fig. 6. 3D Densitogram for calibration curve linearity of ESMO and LEVO

LOD and LOQ: LOD and LOQ were found to be for LEVO was 95.30 ng/band and 641.04 ng/band, for ESMO 31.44 ng/band and 211.54 ng/band and for indicating good sensitivity of the method.

Precision: Method is precise as results for intra-day was found to be for LEVO 1.29-1.73 and for ESMO 0.02-0.09, while for inter-day it was found to be for LEVO 1.45-1.61 and for ESMO 0.19-1.53. Thus the method was found to be precise.

Accuracy: Percentage recovery rates of ESMO was in range of 198.45-105.07 % and for LEVO, it was between 97.52-99.29 %. Thus the method is accurate.

Specificity: Chromatogram of pharmaceutical formulation obtained using the developed method showed only two peaks at R_f of 0.70 and 0.32 for ESMO and LEVO, respectively and was found to be at the same R_f for both standard drugs. Peak purity of both drugs in marketed dosage form was confirmed by comparing overlaid spectra at the peak start, peak apex and peak end positions of the band. Results shown in Table-6 demonstrate that the purity exceeded 0.999 for all peaks, indicating specificity of method in the presence of various excipients (Fig. 7). Hence, the method was found to be specific in the presence of various excipients and degradation product as revealed from (Figs. 8 and 9).

Robustness: Results presented in Table-6 indicate that selected factors remained unaffected by small variation of these parameters.

Analysis of marketed dosage form: Capsule formulations SOMPRAZ L was analyzed and showed separated peak at R_f value of 0.65 for ESMO and 0.24 for LEVO in the chromatogram of Capsule formulation indicating no interference of the excipients. The RSD value was found to be less than 2.

Conclusion

Both esomeprazole (ESMO) and levosulpiride (LEVO) drugs are susceptible to degradation under acid, alkali, UV light and sunlight. The full factorial (FFD) design and response

TABLE-6
ROBUSTNESS DATA FOR LEVO AND ESMO

Drugs	Ratio	R_f	Area ± S.D (ng/band)	RSD
Change in mobile phase ratio: Ethyl acetate:methanol:toluene:ammonia (7:1.5:1.5:0.1v/v/v/v) [a] ± 0.2 mL				
LEVO	6.8:1.5:1.3:0.1	0.32 ± 0.02	2431.0 ± 24.53	1.00
	7.2:1.5:1.7:0.1	0.32 ± 0.02	2228.8 ± 26.49	1.18
ESMO	6.8:1.5:1.3:0.1	0.70 ± 0.02	7111.6 ± 105.75	1.48
	7.2:1.5:1.7:0.1	0.70 ± 0.02	7354.2 ± 86.35	1.17
Change in chamber saturation time (20 min ± 5) [a]				
Drugs	Saturation Time	R_f	Area ± S.D (ng/band)	RSD
LEVO	15 min	0.32 ± 0.02	2891.1 ± 7.2	0.25
	25 min	0.32 ± 0.02	1152.4 ± 5.50	0.48
ESMO	15 min	0.70 ± 0.02	7517.9 ± 79.17	1.05
	25 min	0.70 ± 0.02	7520.7 ± 63.14	0.84
Change in Distance travel (8.5 cm ± 1) [a]				
Drugs	Distance Travel	R_f	Area ± S.D (ng/band)	RSD
LEVO	8 cm	0.32 ± 0.02	2012.1 ± 32.60	1.62
	9 cm	0.32 ± 0.02	2316.9 ± 45.62	1.96
ESMO	8 cm	0.70 ± 0.02	7207 ± 68.96	0.94
	9 cm	0.70 ± 0.02	7265.1 ± 53.53	0.73
Change in wavelength (254 nm ± 2) [a]				
Drugs	Wavelength	R_f	Area ± S.D (ng/band)	RSD
LEVO	252	0.32 ± 0.02	1507.8 ± 8.96	0.59
	256	0.32 ± 0.02	1747.2 ± 21.41	1.22
ESMO	252	0.70 ± 0.02	6469.0 ± 29.03	0.44
	256	0.70 ± 0.02	6895.6 ± 134.46	1.95
Scanning time interval [a]				
Drugs	Hour	R_f	Area ± S.D (ng/band)	RSD
LEVO	1 h	0.32 ± 0.02	3320.9 ± 49.79	1.49
	2 h	0.32 ± 0.02	3797.3 ± 45.01	1.18
ESMO	1 h	0.70 ± 0.02	8668.0 ± 101.44	1.17
	2 h	0.70 ± 0.02	8825.3 ± 137.59	1.55

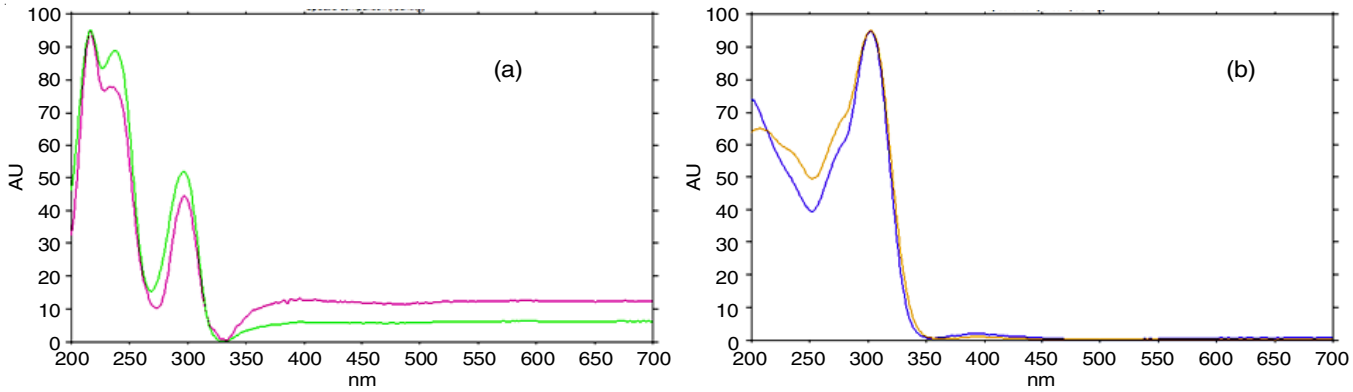

Fig. 7. Overlain peak purity spectra of (a) LEVO and (b) ESMO with the corresponding standard

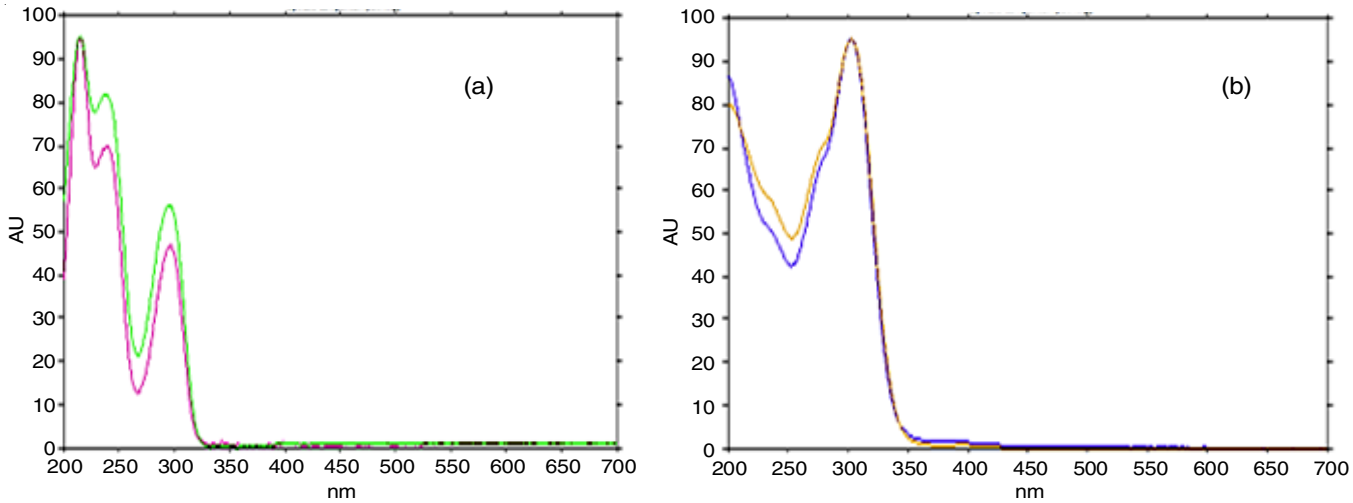

Fig. 8. Overlain spectra of acid induced degradation sample with standard showing peak purity (a) LEVO and (b) ESMO

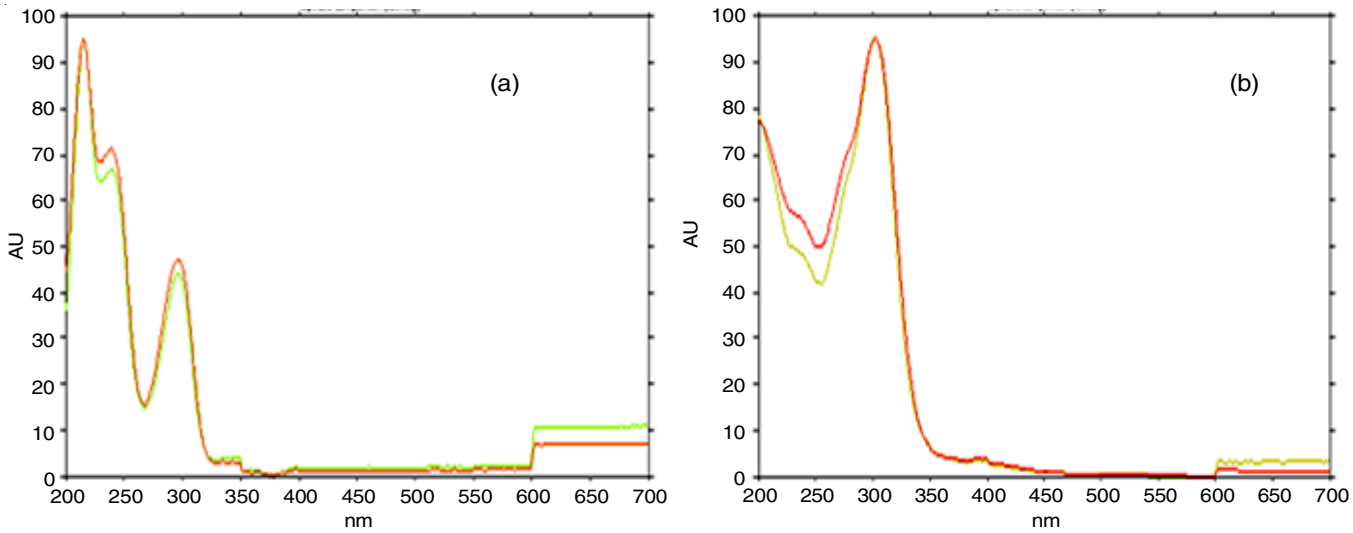

Fig. 9. Overlain spectra of base induced degradation sample with standard showing peak purity (a) LEVO and (b) ESMO

surface methodology helps to obtained essential information on stability of ESMO and LEVO to various degradation conditions. The temperature, time and normality were simultaneously optimized by applying a useful experimental design tool, response surface methodology and derringer's desirability function. The obtained results indicated that the use of a FFD 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. Developed experimental design approach would helpful to determine degradation of formulation at any dependent variable in desirable range. Hence, it is more helpful for the further research compare to conventional approach. Furthermore, it is an economic method that can be used to generate a maximum amount of information in less time with a small number of experiments, as number of sample analyzed and rarely require

clean up. Lower expenditure of solvent pur-chase and disposal since the required amount of mobile phase per sample is small.

Methodological validation indicates that the established HPTLC method is accurate and suitable for the rapid quantitative analysis of ESMO and LEVO in routine QC analysis. The proposed HPTLC method can be successfully utilized to simultaneously estimate the amounts of ESMO and LEVO in pharmaceutical dosage form without interference.

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