SHORT COMMUNICATIONS

DEVELOPMENT, OPTIMIZATION AND VALIDATION OF HPLC METHOD FOR ASSAY AND DISSOLUTION TESTING OF FUROSEMIDE TABLET FORMULATION UTILIZING DESIGN OF EXPERIMENT APPROACH

ABSTRACT

A robust HPLC method was developed and validated for the quantitative estimation of furosemide tablet and dissolution testing. The optimization of chromatographic conditions (% organic phase, flow rate and pH of aqueous phase) was done using the Box-Behnken experimental design. The creation of a reliable HPLC technique utilised the design of experiments methodology effectively. The furosemide peak was found to have a Rt value of 4.86 min under the optimal HPLC settings, which included a C18 (250 mm 4.6 mm, 5 m) column and a methanol: aqueous solution of formic acid buffer (0.2 % V/V, pH 2.5) as mobile phase.The optimized method was validated according to ICH Q2(R1) guideline. Using a fractional factorial design with six chromatographic components, the robustness of the approach was evaluated. The results showed that a multivariant approach was successfully used to improve a reliable HPLC technique for everyday quality monitoring of furosemide in tablet formulations.

Keywords: Box-Behnken design, dissolution testing, fractional factorial design, furosemide, HPLC

INTRODUCTION

Furosemide is extensively used as a potent loop diuretic in the treatment of hypertension and edematous states caused by hepatic, renal and cardiac failure[1, 2]. The present study describes DoE supported robust HPLC method for quantitative estimation of furosemide in pharmaceutical formulation. Box–Behnken design (BBD) was used to analyse and calculate the influence of the chosen factors on response[1-5]. Additionally, the developed and improved HPLC method was validated as per ICH guideline recommendations to confirm fitness to its purpose; including a robustness study utilizing a DoE approach employing fractional factorial design (FFD).

MATERIALS AND METHODS

Materials and Instruments

Furosemide was obtained from Relax Pharmaceutical Biotech Ltd, Baroda. Lasix tablets (Furosemide 40mg, Sanofi-Aventis) were purchased from the market. HPLC experiments were performed using HPLC grade methanol, water, acetonitrile, and formic acid, which were acquired from LOBA Chemie Pvt. Ltd. in India and used in HPLC studies. The auto-injector system (LC2010AT Prominence, Shimadzu, USA) with photodiode array detector was used.

Development, optimization and validation of HPLC method

The independent chromatography factors selected were X_1: percentage (%) of methanol (-1 level =50 and +1 level = 70 %w/w); X_2: flow rate of mobile phase (-1 level = 0.5 and +1 level = 1.5 mL min^{-1}) and X_3:pH of aqueous phase (-1 level = 2 and +1 level = 3) and three center points resulting in total 15 experimental runs. Selected models were analyzed for furosemide peak R_t, T_f, and N (responses) respectively using 1000 µg mL^{-1} furosemide stock solution prepared in methanol. According to ICH Q2R1 guidelines, the improved HPLC method was verified.

RESULTS AND DISCUSSION

Adequate results were obtained using C18 column (100A, 250*4.6 mm, 5 µm, Phenomenex) employing the mobile phase in the isocratic mode (Fig. 1A). BBD was used to optimize X_1 (percentage (%) of methanol), X_2 (flow rate of mobile phase) and X_3 (pH of aqueous phase) to evaluate the effects of variables on furosemide peak R_t, T_f and N. Constraints in optimization design were R_t between 4 - 10 mins, T_f between 1 to 1.5, and N > 2000. The higher value of correlation coefficients (0.9227 for R_t; 0.9112 for T_f; and 0.9811 for N) signifies an excellent correlation between the independent variables indicating the suitability of the chosen models. All of the aforementioned factors suggest that the regression model is very adequate. The mathematical relationships for the measured responses that were produced by software in the form of a polynomial equation, R_t, T_f, and N are

shown below as equations 1, 2 and 3, respectively. $R_t = 4.92 - 0.79\ X_1 - 1.05X_2 + 0.29X_3 + 0.21X_1X_2 - 0.56X_1X_3 - 0.02X_2X_3 + 0.04X_1^2 + 0.52X_2^2 - 0.31X_3^2$ (1); $T_f = 1.13 + 0.18X_1 - 0.08X_2 + 0.14X_3 - 0.15X_1X_2 + 0.07X_1X_3 - 0.18X_2X_3 + 0.01X_1^2 + 0.01X_2^2 - 0.2X_3^2$ (2); and $N = 2198.67 - 370.88\ X_1 + 67.75X_2 + 466.13X_3 + 42.00X_1X_2 - 792.25X_1X_3 + 72.0X_2X_3 - 169.71X_1^2 + 344.04X_2^2 + 197.79X_3^2$ (3). The interactions between independent variables (X1, X2, and X3) and their effects on the responses Rt, Tf, and N are depicted in equations 1 through 3, respectively (Fig. 1B). A positive sign denotes an additive impact, whereas a negative sign denotes a diametrically opposed effect. Desirability one, which provided the best chromatographic conditions of 60% V/V methanol composition, 1.0 mL min^{-1} flow rate, and pH of aqueous phase 2.5, was obtained based on the criteria for furosemide peak Rt, Tf, and N. Replicate injections (n=6) of the furosemide standard sample at 100 µg mL^{-1} were examined to verify and confirm these indicated chromatographic conditions. The results revealed that the observed and anticipated furosemide peak Rt, Tf, and N values varied by less than 5%. The creation of a reliable HPLC method used the DoE approach successfully. A better separation was attained using the C18 column, mobile phase made up of methanol: formic acid (0.2% V/V, pH 2.5) (60:40 V/V), flow rate 1.0 mL min^{-1}, injection volume 20 µL, temperature 25 °C (column oven), and wavelength of 275 nm. Furosemide peak was separated at Rt value 4.86 min using proposed method, as shown in Fig. 1A.

According to the results of the stress testing experiments, the approach was very specific for

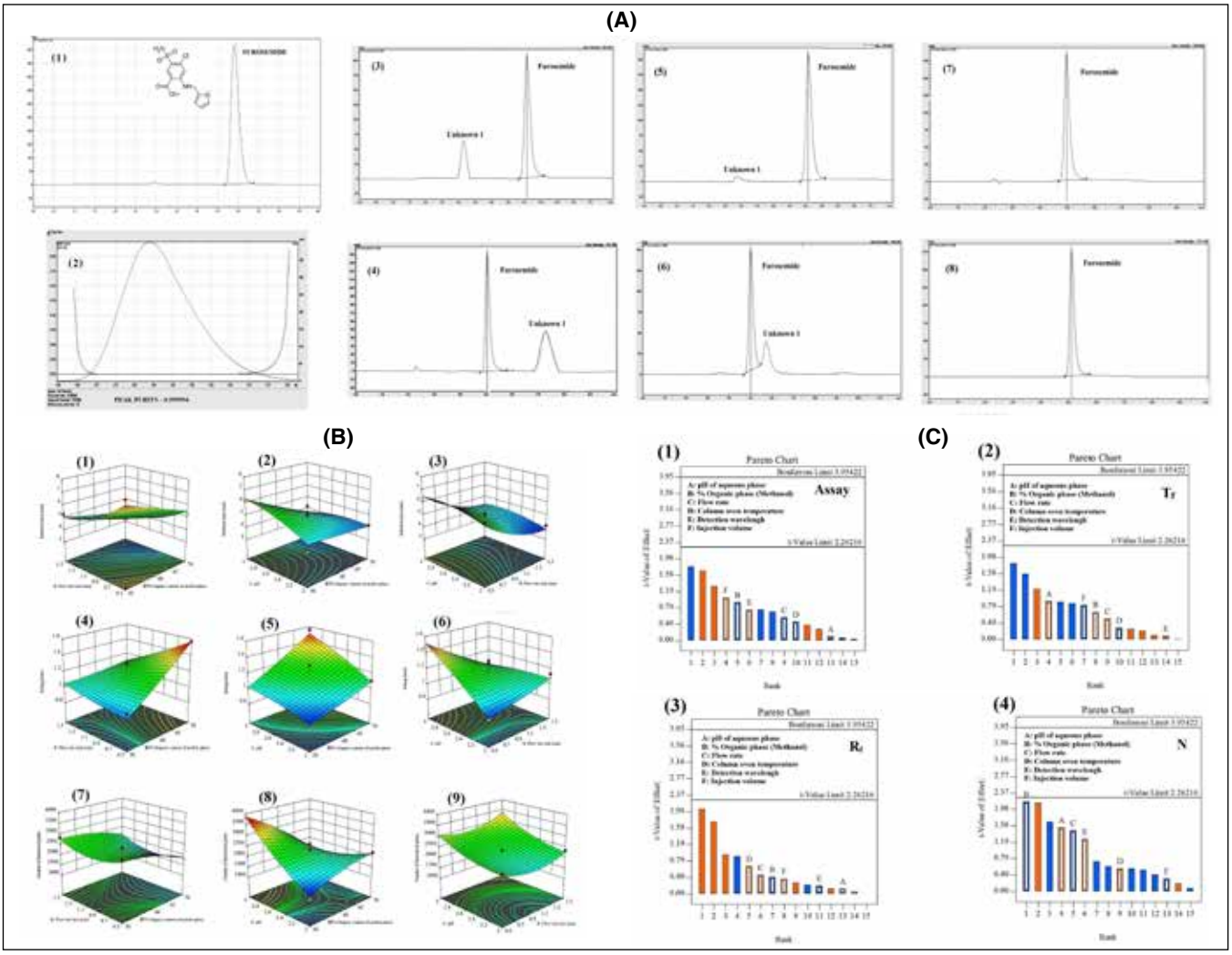

Fig. 1: (A) Representative chromatogram of furosemide (1) and peak purity curve (2), chromatograms for furosemide after forced degradation study using (3) acidic (4) basic (5) thermal (6) moisture (7) photolytic and (8) oxidative stress conditions, respectively using proposed HPLC method; (B) Response surface plots showing the effect of X_1, X_2, and X_3 R_t, T_f and N, respectively; (C) Pareto charts representing the effects of the robustness variables on (1) assay, (2) T_f, (3) R_t and (4) N, respectively, obtained using fractional factorial experimental design investigation

furosemide based on its possible degradation products. Furosemide was discovered to decay in acidic, basic, temperature, and moisture environments, as indicated in Fig. 1A. The furosemide was entirely isolated from the breakdown products. The furosemide peak was devoid of any co-eluting material, indicating that the proposed HPLC technique is specific for the quantitative measurement of furosemide, and no interference from formulation excipients was detected either. By performing regression analysis on data acquired for furosemide across the concentration range of 50-150 µg mL^{-1}, the linearity of the improved HPLC technique was demonstrated. LOD of furosemide was 4.28 µg and LOQ was 12.96 µg, respectively. Recovery results were found in the range from 100.11-100.60% w/w of furosemide, using the proposed HPLC method. The proposed HPLC method displayed %RSD for interday and intraday precision in the range of 0.01-0.19 and 0.02-0.15, respectively (Table I). There was no significant effect of deliberate change in the values of all six factors on selected responses including furosemide tablet assay, R_t, T_f and N, respectively, confirming the robustness of the HPLC method (Fig. 1C).

Table I: Summary of validation parameters

Parameter	Furosemide
Linear regression analysis	
Slope	68952
Y-intercept	607000
R square	0.994
Sensitivity	
LOD	4.28 µg
LOQ	12.96 µg
Accuracy, %	
80%	100.11
100%	100.51
120%	100.60
Precision (RSD, %, n=6)	
Intermediate precision (interday)	0.01-0.19
Repeatability (intraday)	0.02-0.15
Specificity	Specific for furosemide
System suitability	
Retention time	4.86 ± 0.2
Area (average)	7049857 ± 7728
Number of plates	2833 ± 23
USP tailing factor	1.44
Repeatability	1.69
Peak purity	0.9999

CONCLUSION

A simple, specific, and robust HPLC method has been developed, optimized, and validated for quantitative estimation of furosemide tablet (98.28–100.44% with % RSD less than 2) and dissolution testing (% cumulative release was found to be more than 80 % in 60 mins) employing the DoE approach. The optimized method was validated according to ICH Q2(R1) guideline for specificity (peak purity >0.9999), linearity (50-150 µg mL^{-1}), sensitivity (LOD – 4.28 µg; LOQ – 12.96 µg), accuracy (100.11 – 100.51 %), precision (< 2 % RSD), and robustness.

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