

Design of Experiment Approach for the Development and Validation of a Stability-Indicating High-Performance Thin-Layer Chromatography Method for the Estimation of Metformin Hydrochloride and Ursodeoxycholic Acid in Pharmaceutical Dosage Form

Rajendra K. Patel* and Vihari K. Shah

Key Words:

Metformin hydrochloride
Ursodeoxycholic acid
Box–Behnken design
Design of experiment

Summary

In the present study, a design of experiment (DoE) approach was used to optimize chromatographic conditions for the development of a high-performance thin-layer chromatography (HPTLC) method for the simultaneous estimation of metformin hydrochloride (MET) and ursodeoxycholic acid (URSO) in pharmaceutical dosage form. The critical factors were identified using a Taguchi design, and after identification of critical factors, optimization was done using Box–Behnken design (BBD). BBD was used to optimize the compositional parameters and to evaluate the main effect, interaction effects, and quadratic effects of the mobile phase compositions, development distance, and saturation time on the retardation factor (R_f) of both drugs. HPTLC separation was performed on aluminum plates pre-coated with silica gel 60 F₂₅₄ as the stationary phase, using toluene–ethanol–acetone–formic acid (4.5:2:2.5:0.85, V/V) as the mobile phase at a wavelength of 234 nm and 700 nm for MET and URSO, respectively. A sharp and well-resolved peak was obtained for MET and URSO at R_f values of 0.19 and 0.80 min, respectively. The calibration curve was in the range of 5000–40000 ng per spot and 1500–12000 ng per spot for MET and URSO, with $r^2 = 0.984$ and $r^2 = 0.980$, respectively. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantification, and specificity. To provide a better visualization of the statistically significant factors derived from the statistical analysis, the perturbation plot and response surface plot for the effect of independent variables on the R_f of MET and URSO were evaluated. Stability study was performed under different stress conditions such as acid and alkali hydrolysis, oxidation, and temperature. The developed method was able to resolve drugs and their degradation products formed under the afore-mentioned conditions.

1 Introduction

Diabetes mellitus type 2 is generally known as non-insulin dependent diabetes mellitus (NIDDM) or also as adult-onset diabetes. It is a metabolic issue involving high blood glucose (hyperglycemia) in the setting of insulin resistance and relative insulin lack [1]. Diabetes mellitus is difficult to control with a single oral hypoglycemic agent, and the rate of monotherapy failure is high. Hence, combination therapy with complementary classes of drugs that act on different aspects of glycemic control would be expected to be an effective strategy for the control of diabetes. Metformin hydrochloride is a biguanide hypoglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus. Metformin hydrochloride has the chemical name of 1,1-dimethylbiguanide hydrochloride, a structural formula of $C_4H_{11}N_5 \text{ HCl}$. It is effective in glycemic control and decreases the intestinal absorption of glucose. Metformin hydrochloride is freely soluble in water, slightly soluble in methanol and ethanol, and practically insoluble in acetone and chloroform. Ursodeoxycholic acid (URSO) (3 α ,7 β -dihydroxy-5 β -cholan-24-oic acid), a structural formula of $C_{24}H_{40}O_4$, also known as ursodiol, is a naturally occurring bile acid (BA). BAs are steroid compounds, hydroxyl derivatives of 5 β -cholan-24-oic acid. URSO is freely soluble in ethanol (96%) and methanol, and practically insoluble in water and methylene chloride [2–3].

The literature review revealed few methods for the estimation of MET and URSO alone or in combination with other drug by high-performance liquid chromatography (HPLC) [4–9], high-performance thin-layer chromatography (HPTLC) [10–12], liquid chromatography–mass spectrometry (LC–MS) [13–14], and UV spectrophotometric methods [15–16]. However, there are no reported assay methods that permit the simultaneous quantification of MET and URSO in combined dosage form.

The aim of the present work was to optimize the chromatographic conditions using design of experiment (DoE) approach and to develop a simple, accurate, precise, sensitive, and specific stability-indicating HPTLC method for the simultaneous quantification of metformin hydrochloride

R.K. Patel, V.K. Shah, Pharmaceutical Chemistry Department, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat 382023, India.
*E-mail: rajukpatel2006@gmail.com