A Validated Stability Indicating High Performance Thin Layered Chromatographic Method for the Analysis of Dapagliflozin in Bulk Drug and Marketed Tablet Formulation

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Present study reported the development of validated stability indicating high performance thin layer chromatography method for determination of dapagliflozin in bulk and tablet dosage form. Chromatography was performed on aluminium plates coated with silica gel 60F₂₅₄ using methanol:toluene:ammonium acetate (6.9:3:0.1 v/v/v) as mobile phase. Densitometric analysis was performed at 250 nm. The method was validated with different parameters such as linearity, precision, accuracy, specificity, robustness, limit of detection (LOD) and limit of quantitation (LOQ). The R_f value of dapagliflozin was found to be 0.29 ± 0.05. The method is sensitive (limit of quantification 50.5 ng band⁻¹), precise (RSD ≤ 1.50 %), accurate (drug recovery 98.90-100.53 %) and linear over the range 100-1000 ng band⁻¹ (r² 0.9985). The developed method was satisfactorily applied for the analysis of pharmaceutical preparations and found to be specific and accurate for quality control of the dapagliflozin in tablet dosage form.

Keywords: HPTLC, Dapagliflozin, Stability indicating, Validation, Degradation products.

INTRODUCTION

Dapagliflozin (DAPA) belongs to a new class of oral anti-diabetic drugs, called sodium glucose co-transporter 2 (SGLT2) inhibitors. Inhibiting SGLT2, which have a key role in the reabsorption of glucose in the kidney, has been proposed as a novel therapeutic strategy for diabetes. Genetic mutations in the kidney-specific SGLT2 isoforms that results in benign renal glucosuria. Hence it indicates that elevating renal glucose excretion by suppressing SGLT2 can reduce plasma glucose level as well as weight [1,2]. Dapagliflozin was approved by the FDA on 2014, Jan 08. Dapagliflozin is not recommended for patients with diabetes mellitus type 1 or for the treatment of diabetes ketoacidosis. Dapagliflozin is used for the adjunct management of glycemic control in patients with type 2 diabetes mellitus, in combination with diet and exercise [3,4]. It is chemically known as, (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]-phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol (Fig. 1). The molecular formula is C₂₁H₂₅O₆Cl and molecular weight is 408.873.

Dapagliflozin is stable under thermal, photo and neutral hydrolysis stress conditions but it is liable to undergo a degra-

Fig. 1. Structure of dapagliflozin

dation leading to formation of various degradants under the condition of stress like acid and alkali hydrolysis and oxidation. This necessitated test of the drug for stability, furthermore the development of stability indicating assay methods for dapagliflozin. The International Conference on Harmonization (ICH) guideline Q1A (R2) for parent drug stability-indicating test suggests that stress testing on the drug be performed to establish the stability characteristics and to support the suitability of the proposed analytical method [5-7]. Developing an ideal stability-indicating assay method (SIAM) means to design such a test that quantifies a drug and resolves its degradation products with high accuracy, moreover that allows the determination of the drug and its degradants in the presence of the excipients of the formulations.

Various methods have been published for the routine determination of dapagliflozin alone [4,8,9] and in combination with other drugs by means of high-performance liquid chromatography (HPLC) [10] as well as various hyphenated techniques like liquid chromatography-tandem mass spectrometry (LC-MS/MS) [2]. Methods for stability-indicating assays of dapagliflozin are also available by UV [11,12], HPLC [12-14]. However, after literature survey, it was found that there is no report on any SIAM for dapagliflozin by high-performance thin-layer chromatography (HPTLC). HPTLC is advantageous over other methods because many samples can be analyzed simultaneously in very low quantity of sample and solvent, thus, reducing the operational cost and time. In our continuous efforts to develop simple, precise and sensitive methods for the analytical determination of drugs in API and formulations, herein we report the stability-indicating assay method for dapagliflozin. To our best of knowledge, this is the first report regarding the SIAM of dapagliflozin by means of HPTLC in the presence of its degradation products.

EXPERIMENTAL

Dapagliflozin standard is obtained as a generous gift sample from Dr Reddy's Lab Hyderabad, India. Dapagliflozin tablets (Forxiga®) labeled to contain 10 mg, manufactured by Bristol Myers Squibb S.R.L and imported and marketed by Astra Zeneca Pharma India Limited, were purchased from local market (batch No. AAS2563; manufacturing date 02.2017 and expiry date 01. 2020). All other reagents used for experimentation were of analytical reagent (AR) grade. Methanol, toluene and ammonium acetate (AR) were purchased from Merck Specialties Private Limited (Bengaluru, India).

Conditions: The chromatographic separation of the drugs was performed on Merck HPTLC plates precoated with silica gel 60 F254 (10 cm × 10 cm with layer thickness of 150–200 µm, with surface thickness deviation of ≤ 30 µm, E. Merck [Merck Millipore, Darmstadt, Germany]). The samples were applied onto the plates as bands with 5-mm width using a CAMAG (Muttens, Switzerland) 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) with a CAMAG Linomat-IV TLC sample applicator. Linear ascending development was carried out in a twin-trough glass chamber (10 cm × 10 cm). Densitometric scanning was performed using a CAMAG TLC Scanner III (version 4.0.1) supported with CAMAG winCATS® software (version 4.0.1). An electronic balance (ACCULAB Model ALC-210.4 Huntington Valley, PA, USA), a sonicator (EN 30 US, Entertech Fast-clean, Mumbai, India) and a photostability chamber (Desaga, Sarstedt Gruppe, Wiesloch, Germany) were also used.

A mixture of methanol:toluene:ammonium acetate in the ratio of 6.9:3:0.1 (v/v/v) was optimized for thin-layer chromatographic plate development. The chamber was saturated with the mobile phase at room temperature for 15 min. The run distance was kept at ~ 70 mm and 10 mL of the mobile phase was used for a single development. The dosing speed of nitrogen applicator was kept 150 nL s⁻¹ with a pre-dosage volume of 5 mL. The samples were applied as bands of 5 mm width with gaps of 5 mm in-between. The developed plates were dried at room temperature for 5 min; detection was done at 250 nm using a

deuterium lamp in absorption–reflectance mode and the slit dimension of the detector was kept at 4 mm × 0.45 mm.

Method validation: Developed HPTLC method was validated on the basis of following parameters:

Linearity: Dapagliflozin in different concentrations (100–1000 ng band⁻¹) were applied as a band of 5 µL on the HPTLC plate and the peak areas were measured on the densitometer. The calibration curve was constructed by plotting peak area *versus* applied concentration. The most convenient way of linearity besides a visual assessment is plotting residuals. The regression equation was constructed with each response as an average of three determinations.

Precision: Intra-day precision was evaluated by performing replicate analysis (n = 6) of QC samples (200, 400 and 600 ng band⁻¹). Inter-day precision was determined by repeating the intra-day assay on 3 different days. Precision was expressed as the % RSD of the measured concentration for each calibration level.

Accuracy: The accuracy of the method was determined on the basis of recovery studies performed by standard addition at different levels of the label claim. A known amount of standard was added to samples of tablet powder, which was then mixed, extracted and subsequently diluted to volume with AR-grade methanol, to yield the required concentration of drug.

Limit of detection and limit of quantitation: As per ICH guideline, limit of detection (LOD) and limit of quantitation (LOQ) of the developed method was calculated from standard deviation of the response and slope of the calibration curve, limit of detection = $3.3 \times \sigma/S$ and limit of quantitation = $10 \times \sigma/S$, where, σ is standard deviation of response and S is the slope of calibration curve.

Specificity: Specificity was performed using the typical constituted placebo, blank solvent used for the analysis of active pharmaceutical ingredient (API). The placebo degraded under the same conditions as applied for API.

Analysis of dapagliflozin in marketed tablet formulation: An accurately weighed quantity of tablet powder equivalent to 10 mg of dapagliflozin was transferred to a dry, clean 50 mL volumetric flask. 20 mL of methanol was added and the mixture was sonicated for 30 min. The solution was kept at room temperature for 5 min and volume was made up to the mark with methanol. The resulting solution was filtered through Whatman grade I filter paper. Transfer exactly 4 mL of the filtrate to 10 mL of volumetric flask and dilute up to the mark with methanol (400 ng band⁻¹). The resulting solution was applied to a plate along with standard for analysis.

Force degradation study: To carry out the stress study, 10 mg/mL solution of dapagliflozin was prepared in methanol. The hydrolytic degradation studies of dapagliflozin were carried out at a drug concentration of 1 mg mL⁻¹ in 1 N HCl, 1 N NaOH and water at 60 °C till sufficient degradation was achieved. Dapagliflozin was dissolved at a concentration of 1 mg mL⁻¹ in 20 % H₂O₂ and kept at room temperature for oxidative stress study. The drug powder was spread on a petri plate and kept under sunlight and UV light for 7 days to determine the effect of photolytic conditions. Dapagliflozin in powder form was kept at 60 °C for 7 days to study the effect of heat.

RESULTS AND DISCUSSION

Mobile phase optimization: The stressed samples were initially analyzed by HPTLC using the mobile phase toluene: methanol (5:5 v/v); the peak was observed at a retardation factor (R_f) of 0.56. However, the symmetry of this peak was not proper due to the fronting effect. In order to improve the results, individual and combination of varying polarity of solvents were tried in order to get R_f value in the range from 0.2-0.7 for dapagliflozin and separated degradation products. The mobile phase comprising of methanol:toluene:ammonium acetate (6.9:3:0.1 v/v/v) was found to be adequate for the same. The retardation factor of dapagliflozin was about 0.29 ± 0.05 (Fig. 2).

Fig. 2. Densitogram of dapagliflozin (500 ng band⁻¹)

Method validation: The response for the drug was found to be linear in the concentration range of 100-1000 ng band⁻¹ for dapagliflozin drug substance with a correlation coefficient of 0.9985. The regression equation was obtained as $y = 6.4138x + 1079.9$, where y is the peak area and x is the concentration (ng band⁻¹) (Table-1). The linear calibration, calculated with linear regression and residuals were plotted, proving the linearity as shown in Fig. 3. The results of intra-day and inter-day precisions are listed in Table-2. Intra-day precision was found to be $(n = 6) \leq 1.42\%$ and inter-day precision was calculated as $\leq 1.52\%$. Repeatability was found to be 1.50% which confirms that the method is precise. The results of the accuracy study are shown in Table-3. Recovery was in the range of 98.90-100.53 %, indicating the accuracy of the method. The LOD and LOQ were found to be 16.7 and 50.5 ng band⁻¹, respectively. The specificity of the method was ascertained by the absence of any other peak of the placebo, degradation products of the placebo and the solvent. The developed method was found to be robust Table-4. A summary of the validation parameters is given in Table-5.

TABLE-1
CALIBRATION DATA FOR LINEARITY

Amount (ng/band)	Peak area $\pm$ % RSD at 250 nm (n = 3)
100	1725.0 $\pm$ 1.88
200	2355.7 $\pm$ 0.79
400	3671.0 $\pm$ 1.22
600	4936.7 $\pm$ 0.78
800	6240.0 $\pm$ 0.22
1000	7474.7 $\pm$ 0.43

n = Number of replicates

Fig. 3. Linear calibration, calculated with linear regression. Residuals prove linearity

TABLE-2
PRECISION OF THE ANALYTICAL METHOD

Amount (ng band ⁻¹)	Peak area $\pm$ % RSD (n = 3) Intra-day	Peak area $\pm$ % RSD (n = 3) Inter-day
200	2334.66 $\pm$ 1.42	2366.33 $\pm$ 1.16
400	3667.86 $\pm$ 1.32	3672.12 $\pm$ 0.75
600	4996.65 $\pm$ 0.87	4511.05 $\pm$ 1.52

TABLE-3
RECOVERY STUDY OF THE ANALYTICAL METHOD

Test conc. (ng/band)	Amount spiked (ng/band)	Amount recovered (ng/band)	% Recovery $\pm$ % RSD
100	100	198.3	99.15 $\pm$ 1.59
100	300	390.6	98.90 $\pm$ 1.71
100	500	603.2	100.53 $\pm$ 1.65

TABLE-4
RESULTS OF ROBUSTNESS STUDY

Conditions	Modifications (methanol:toluene: ammonium acetate)	Mean area ^a $\pm$ % RSD
Mobile phase composition	7.1:2.8:0.1	3799 $\pm$ 1.36
	6.9:3.0:0.1	3722 $\pm$ 0.90
	6.7:3.2:0.1	3432 $\pm$ 0.89
Amount of mobile phase (mL)	10.7	3621 $\pm$ 0.79
	10.2	3689 $\pm$ 1.52
	9.7	3711 $\pm$ 1.42
Chamber saturation time (min)	20	3672 $\pm$ 1.36
	30	3631 $\pm$ 1.11
	40	3720 $\pm$ 0.88

^aAverage of three replicates

TABLE-5
SUMMARY OF VALIDATION PARAMETERS

Validation parameter	Dapagliflozin
Regression equation	$Y = 6.4138x + 1079.9$
Correlation coefficient	0.9985
Linearity	100–1000 ng band ⁻¹
Precision (intra-day)	$\leq 1.42\%$
Precision (inter-day)	$\leq 1.52\%$
Recovery	98.90-100.53 %
LOD	16.7 ng band ⁻¹
LOQ	50.5 ng band ⁻¹
Specificity	The method is specific
Robustness	The method is robust
Assay	99.58 $\pm$ 1.50 %
R_f	0.29 $\pm$ 0.05

Forced degradation studies: Samples obtained from forced degradation were chromatographed with the optimized mobile phase and it was found that densitogram obtained after acidic hydrolysis gave three degradation products of dapagliflozin at R_f value 0.20 ± 0.03 (DP-I), 0.68 ± 0.03 (DP-II) and 0.72 ± 0.03 (DP-III) (Fig. 4), alkaline hydrolysis gave two degradation products of dapagliflozin at R_f value 0.20 ± 0.03 (DP-I) and 0.72 ± 0.03 (DP-III) (Fig. 5) and oxidation gave degradation product of dapagliflozin at R_f value 0.11 ± 0.03 (DP-IV) (Fig. 6). No degradation products were obtained after neutral, photo and heat stress condition. Percentage degradation was calculated which is listed in Table-6. The drug was found to be more liable to decompositions in acidic, alkaline medium than in oxidative condition. The drug is stable to photolytic and thermal stress conditions.

Fig. 4. Degradation of dapagliflozin (DAPA) in 1 N HCl

Fig. 5. Degradation of dapagliflozin (DAPA) in 1 N NaOH

TABLE-6
DEGRADATION BEHAVIOUR OF DAPAGLIFLOZIN

Stress conditions	Degradation (%)
1 N NaOH (reflux at 60 °C for 1 h)	16.8
1 N HCl (reflux at 60 °C for 1 h)	14.2
Water (reflux at 60 °C for 5 h)	Negligible
20 % H ₂ O ₂ (room temperature for 3 h)	9.6
Photolysis (under UV for 7 days)	Negligible
Thermal (in oven at 60 °C for 7 days)	Negligible

Fig. 6. Degradation of dapagliflozin (DAPA) in 20 % H₂O₂

Conclusion

A new analytical method has been developed to determine dapagliflozin in pharmaceutical dosage form. In this study, the stability of dapagliflozin was established under stress conditions recommended the International Conference of Harmonization guideline. The developed method was proven to be linear, precise, accurate and specific.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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