

A NEW HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop a new simple, selective and precise high-performance thin-layer chromatographic method for determination of Dapagliflozin (DAPA) in bulk and tablet dosage form.

Methods: The present study describes the development and validation of a High-performance thin-layer chromatographic method for DAPA. The chromatographic separation was carried out on Merck precoated silica gel aluminum plate 60 F₂₅₄ using Chloroform: Methanol (9:1v/v) as mobile phase. Quantitative determination of drug was carried out by densitometric scanning of plates at 223 nm using Camag TLC Scanner.

Results: The chromatographic condition showed a compact band with the retention factor for dapagliflozin as 0.21±0.004. The method was validated as per ICH guidelines for linearity, accuracy, precision and robustness. Response was found to be linear in the concentration range of 400 ng/band to 1200 ng/band with linear regression value of 0.9953 with respect to peak area and concentration value. The LOD and LOQ were found to be 1.2083 ng/band and 3.6616ng/band. The percentage assay was found to be 100±0.05.

Conclusion: This method under statistical analysis proved a selective, repeatable and accurate analysis of the drug. This method can be used for quantitative analysis of dapagliflozin in the bulk drug and in tablets as the run time is very minimal.

Keywords: Dapagliflozin, HPTLC, Method development, Validation

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INTRODUCTION

Dapagliflozin (DAPA) is a drug of gliflozin class. Chemically it is (2S, 3R, 4R, 5S, 6R)-2-(4-chloro-3-(4-ethoxybenzyl) phenyl)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol (fig. 1) with molecular formula of C₂₁H₂₅ClO₆ and molecular weight of 408.875g/mol. It is a white crystalline solid, having a solubility in organic solvents like DMSO dimethyl formamide and ethanol. Its melting point is 55-60 °C [1]. Dapagliflozin is a selective sodium-glucose co-transporter subtype 2 (SGLT2) inhibitor with anti-hyperglycemic activity. Dapagliflozin selectively and potently inhibits SGLT2 compared to SGLT1, which is the co-transporter of glucose in the gut. Dapagliflozin blocks glucose reabsorption into the kidney, resulting in the elimination of blood glucose through the urine. DAPA is not official in any Pharmacopoeia as of now.

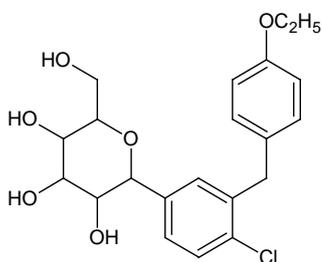


Fig. 1: Chemical structure of Dapagliflozin

The literature survey revealed that very few spectroscopic [2, 3], RP-HPLC [4-13] methods and LCMS/MS [13] methods were reported for DAPA. No HPTLC method was reported in the literature so far for this drug in commercial pharmaceutical preparations. However, few methods were developed for the combination of drugs. A

comparative evaluation of HPLC and HPTLC estimation was carried out for simultaneous determination of dapagliflozin and metformin hydrochloride [14] and UV-spectroscopic first derivative and HPTLC methods were done to estimate dapagliflozin propanediol monohydrate and saxagliptin hydrochloride [15]. This necessitates the requirement for the development of newer methods of analysis for DAPA so that it can be used in routine analysis. The present investigation aimed to develop and validate a method for estimation of DAPA in pharmaceutical dosage forms by HPTLC.

MATERIALS AND METHODS

Chemicals and reagents

Dapagliflozin (DAPA) was a generous gift from Micro Labs, Bengaluru, India and used as such. Chloroform, water and methanol of HPLC grade (Merck Ltd., Mumbai, India) were used. The pharmaceutical dosage form used in this study was a tablet, which was purchased from local retail pharmacy Bengaluru, India. (Trade name OXRA-10 mg/tab manufactured by Sun pharma).

Camag HPTLC system (Camag, Muttenz, Switzerland) consisting of a Camag Linomat V semiautomatic spotting device under nitrogen gas flow and Camag TLC scanner III with WinCATS 4 software (version 4.05, Camag), 100 µL HPTLC sample syringe (Hamilton, Bonaduz, Switzerland), Camag UV chamber and a Camag glass twin-trough (10 cm ×10 cm) development chamber were used. Pre-coated silica gel 60 F₂₅₄ aluminium plate with 75-125 µm layer thickness, 10x10 cm; Merck Germany was used as stationary phase. TLC scanner III with winCATS software was used to carry out the densitometric analysis.

Optimization of HPTLC method

Stock solution

100 mg of DAPA was weighed and dissolved in methanol and the volume was made up to 100 ml to obtain a concentration of 1

mg/ml. From this 1 ml was diluted to 10 ml, which was used as working standard solution.

Sample solution

Tablets was powdered and powder equivalent to 100mg of DAPA was weighed accurately, transferred into volumetric flask, sonicated, sonicated for half an hour with 20 ml of methanol and diluted to a volume of 100 ml to obtain a concentration of 1 mg/ml. From this 1 ml was diluted to 10 ml, which was used as a standard working solution.

Stationary phase

Merck TLC plates Silica gel 60F (10 x10 cm)

Selection of mobile phase

Mobile phase: Trial chromatographic runs were carried out using 8 μ l of standard solution when spotted on TLC plate using CAMAG LINOMAT V using nitrogen gas flow to give a concentration of 800

ng/band. The chromatogram was developed in twin trough chamber saturated with mobile phase till the solvent front reached about 7.0 cm. The plate was removed, air-dried and visualized under CAMAG UV chamber. A good resolution with a perfect band was found with Chloroform: Methanol in the ratio of 9:1v/v.

Selection of analytical wavelength

The standard drug solution of DAPA prepared in methanol was scanned in the range of 200-800 nm. The spectrum was recorded. DAPA exhibited maximum absorbance at 223 nm.

Optimization of chromatographic conditions for HPTLC

Based on the above study, it was concluded that the chromatographic separation of DAPA could be achieved under the conditions detailed in table 1. Chromatogram of standard solution recorded under optimized conditions exhibited well-resolved peaks with R_{fvalue} of 0.21 ± 0.004 as shown in the fig. 2.

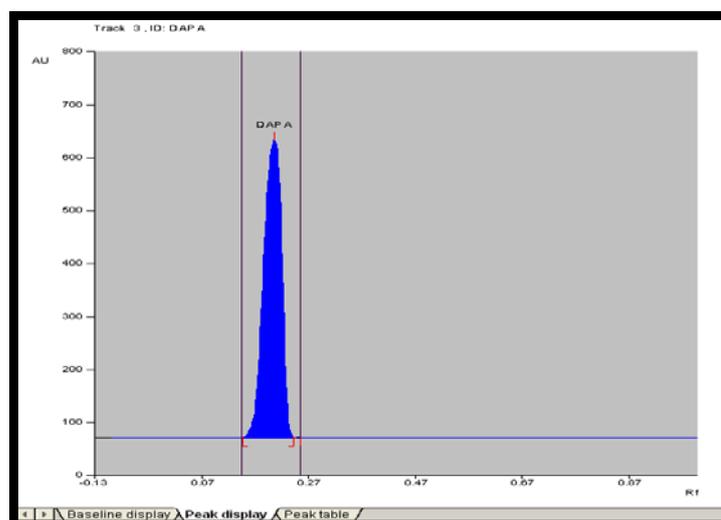


Fig. 2: Densitogram of standard DAPA

Validation parameters of the developed method

Linearity

Five sets of the standard solution of DAPA, 4-12 μ l with 2 μ l increment, was spotted on to the chromatographic plate as described earlier and the chromatogram was developed and scanned at 223 nm. Chromatographic response in terms of area was plotted against concentration and a straight-line fit was made through the data points by least square regression analysis to get linearity plot. From the linearity plot the slope and Y-intercept were noted. Further, the correlation coefficient was also calculated.

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

The LOD and LOQ were calculated using following equations as per International Conference on Harmonization guideline for DAPA.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where σ is standard deviation of the response and S is the standard deviation of y-intercept of regression lines.

Specificity

The chromatogram obtained from the sample run was scanned for peak purity analysis. The peak purity of DAPA was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot. The band DAPA in the sample was confirmed by comparing R_f value and respective spectra of the sample with that of the standard.

Precision (Reproducibility)

The precision of the method was verified by performing the intraday and inter-day precision. The entire linearity range was used to study the precision of the method. Precision of the proposed method was studied with respect to the intraday and inter-day by estimating the corresponding response three times on the same day and on three different days over a period of one week, using the same chromatographic conditions as described above. The results are expressed in terms of relative standard deviation.

Accuracy (Recovery studies)

The accuracy of the method was determined by recovery studies using standard additions at three different levels (80, 100 and 120%) in the formulation. Three determinations were performed at each level, using the same chromatographic conditions as described above.

Robustness

The robustness of the method was studied with an intentional change in the composition of the mobile phase and the values were compared with the original chromatographic conditions. Robustness of the method was determined in triplicate at a concentration level of 800 ng/band and the mean and %RSD of peak area was calculated [16].

Application of the proposed method for the determination of DAPA in tablet

The content of DAPA in marketed tablet dosage form was estimated by the established method. The marketed tablet has the label claim

of 10 mg of DAPA. Twenty tablets were weighed and average weight was determined, powdered, from this equivalent weight of 100 mg DAPA was taken into a 100 ml volumetric flask, containing 15 ml of methanol and sonicated for 30 min, filtered through Whatmann filter paper No.41 and then the volume was made up to 100 ml with methanol. From this 1 ml was diluted to 10 ml, which was used as a working standard solution. From this stock solution 8 μ l containing 800 ng/band was spotted on a HPTLC plate and chromatogram was developed as described earlier. The analysis was repeated for three times and interference for excipients was analyzed.

RESULTS

To optimize the HPTLC parameters for DAPA several mobile phases such as n-hexane: ethyl acetate, chloroform: methanol combination in the varying ratio was tried and the satisfactory result was obtained using the mobile phase chloroform and methanol (9:1 v/v) with UV detection at 223 nm (table 1). A sharp and symmetrical peak was resolved with an Rf of 0.21 \pm 0.004 for DAPA (fig. 2). The method validation results were satisfactory as per ICH Q2B guidelines. The statistical data showed in the table 2 and fig. 3

indicated that linear relationship between peaks area (AUC) and concentration of DAPA standard (ng/band) in the concentration range of 400 ng/band to 1200 ng/band showing a good regression coefficient of 0.9953 (fig. 4, table 3.). Limit of detection and limit of quantification was calculated based on the average value of slope and standard deviation of the y-intercept and was found to be 1.2083 ng/band and 3.6616 ng/band, respectively. The specificity of the developed method was established by analysing the sample solutions containing standard DAPA and marketed tablets in relation to interferences from formulation ingredients (fig. 5). The precision was calculated and was expressed in terms of percentage relative standard deviation and it was found to be 0.02-0.073% for intraday and 0.02-0.06% for inter-day respectively. The results are tabulated in table 4. Percentage recovery in terms of accuracy was found to be within the limit of 98% to 102% (table 5). Robustness was checked by a slight change in the mobile phase composition, saturation time. The method was found to be robust since the peak area values were not significantly affected for DAPA (table 6). The percentage purity is found to be 100 \pm 0.05 average of three trials (table 7).

Table 1: Optimized chromatographic conditions for HPTLC analysis of DAPA

Stationary phase	Merck TLC plates silica gel 60 F 254 (10x10 cm)
Mobile Phase	Chloroform: Methanol (9:1 v/v)
Scanner wavelength	223 nm
Development Chamber	Twin Trough Glass Chamber (10 cmX10 cm)
Band Length and slit dimension	6 mm and 0.5x 0.45 mm
Software	Wincats-version1.4.3
Development Distance	7.0 cm
Tank saturation time	30 min

Table 2: Linearity results for DAPA by HPTLC analysis

Concentration(ng/band)	Area*
400	951
600	1202
800	1376
1000	1565
1200	1745

*Mean of five readings

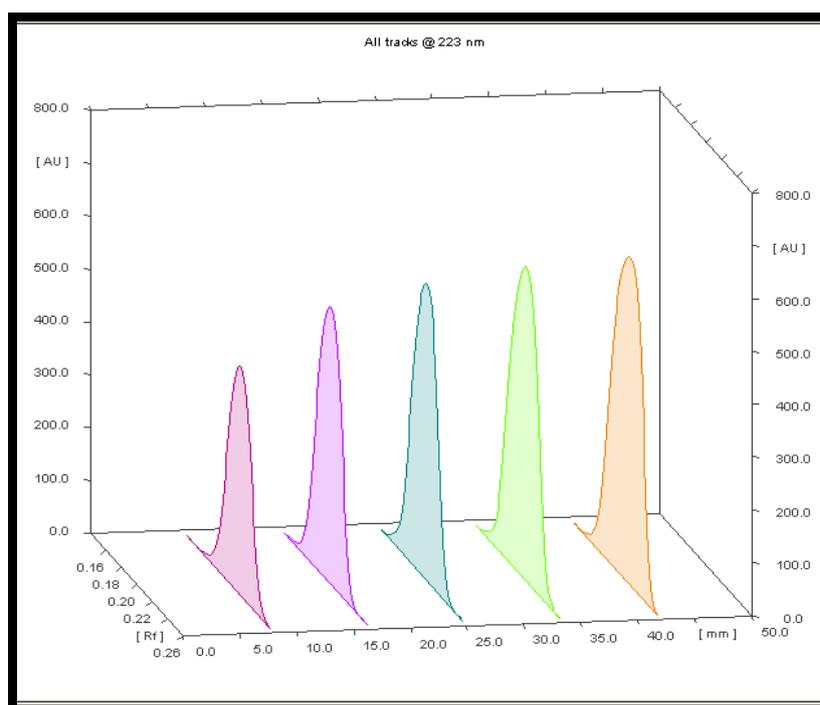


Fig. 3: 3D chromatogram for linearity studies of DAPA

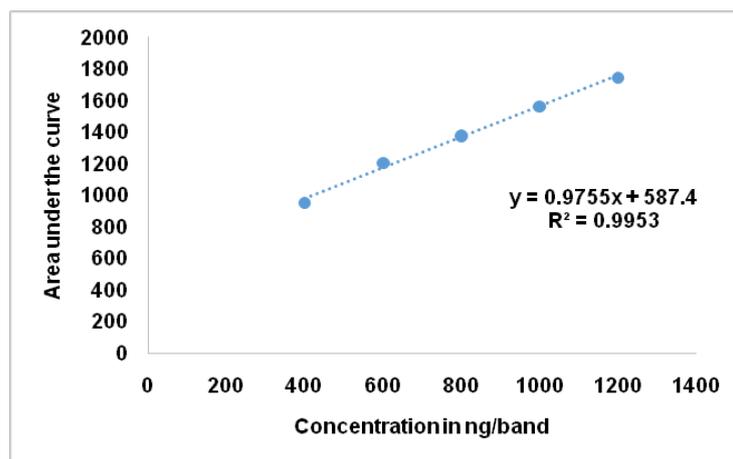


Fig. 4: Linearity curve for DAPA

Table 3: Statistical data of calibration curves of DAPA by HPTLC

Parameters	Results
Linearity Range	400-1200 ng/band
Regression Equation (R ²)	y = 0.9755x+587.4
Correlation Coefficient	0.9953
Slope	0.9755
Intercept	587.4

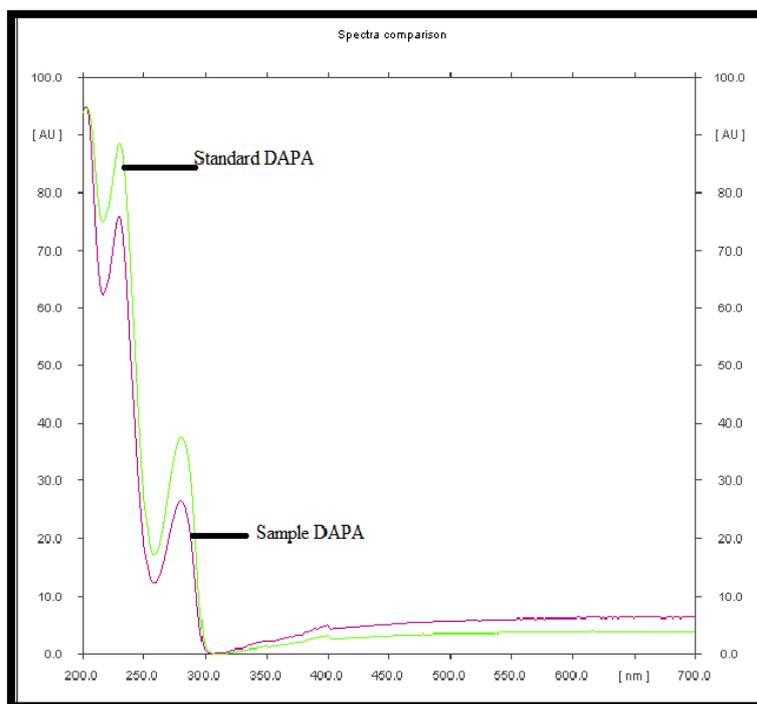


Fig. 5: Specificity of DAPA

Table 4: Precision study of DAPA by HPTLC analysis

No.	Drug level DAPA (ng/band)	Intra day average peak area*	Inter day average peak area*	%RSD for intra day	%RSD for intra day
1	400	952	954	0.020	0.054
2	750	1202	1206	0.045	0.060
3	1000	1375	1389	0.073	0.028
4	1250	1566	1587	0.035	0.074
5	1500	1745	1735	0.054	0.055

*Mean of three readings, %RSD= Percentage Relative standard deviation

Table 5: Recovery studies of DAPA by HPTLC studies

Amount of the drug analysed (ng/band)	Amount of the drug added* (ng/band)	Theoretical calculation*	Total amount of the drug analysed*	% recovery \pm SD*	% RSD
800	640	1440	1440.12	102 \pm 1.7	1.6
800	800	1600	1600.84	99.95 \pm 0.05	0.05
800	960	1760	1760.24	99.98 \pm 0.02	0.02

*mean of three readings, \pm standard Deviation, %RSD= Percentage Relative standard deviation

Table 6: Robustness of the method for DAPA

Parameters	Rf*	Amount* spotted (ng/band)	Amount detected* (ng/band \pm SD*)	%RSD
Chloroform: Methanol 8.9:1.1 v/v	0.21	800	789 \pm 4.04	0.05
Chloroform: Methanol 9.1:0.9 v/v	0.22	8000	801 \pm 5.13	0.06

*mean of three readings, \pm standard deviation, %RSD= Percentage Relative standard

Table 7: Content of DAPA in tablets

Drug	Label claim	Amount found expressed in percentage*
DAPA (OXRA)	10 mg	100 \pm 0.05

*mean of three readings, \pm standard deviation

DISCUSSION

Chromatographic technique and spectrophotometric methods are most commonly used techniques in pharmaceutical analysis. Development of spectrophotometric method involve optimization of fewer parameters whereas development of chromatographic method specifically HPLC method involves optimization of longer number of parameters like mobile phase (type of solvent and their proportion) buffers (type of buffers, pH, buffer strength), peak modifiers (types and concentration), flow rate and isocratic/gradient apart from optimization of detector parameters. Hence conventional trial and error approach will be time-consuming even for experienced analyst. Hence a novel, simple and precise high-performance thin-layer chromatographic (HPTLC) method coupled with densitometer, was developed for the estimation of DAPA in the pharmaceutical dosage form.

DAPA is a selective sodium-glucose co-transporter subtype 2 (SGLT2) inhibitor with anti-hyperglycemic activity. Many spectrophotometric methods [2-3] and chromatographic methods [4-13] have been reported for estimation of DAPA in bulk and formulation. In combination, DAPA has to be analysed by chromatographic methods [14, 15]. A work carried out by Sally *et al.* [14] used acetonitrile: ammonium acetate:10% acetic acid (9:0.9:0.1, v/v) as the mobile phase at 225 nm. In another study reported by Parmar *et al.* [15] carried out for simultaneous estimation of DAPA, propanediol and saxagliptin Hydrochloride (SAXA) using Chloroform: Ethyl acetate: methanol: ammonia (6:2:2:2 Drops) as Mobile Phase and 210 nm as detector wave length, the Rf value of DAPA and SAXA was found to be 0.30 and 0.54 respectively.

No HPTLC method was reported in the literature so far for this drug in commercial pharmaceutical preparations. In the present study, a simple HPTLC method has been developed, The solvent system: Chloroform: Methanol in the ratio of 9:1v/v. of 8 μ l exhibited well-resolved peaks with Rf value of 0.21 \pm 0.004 as shown in the fig. 2 at 223 nm when carried under optimized conditions as listed in the table 1. The statistical data showed a good linear relationship in the concentration range of 400 ng/band to 1200 ng/band with a regression coefficient of 0.9953. The LOD and LOQ was found to be 1.2083 ng/band and 3.6616 ng/band respectively. The precision in terms of intraday and inter-day was calculated and was expressed in terms of percentage relative standard deviation and it was found to be 0.02-0.073% for intraday and 0.02-0.06% for inter-day respectively and was found to be within standard value as shown in the table 4. The low values of %RSD showed that the method is precise. Percentage recovery in terms of accuracy was found to be within the limit of 98%to 102% as tabulated in the table 5.

Robustness was checked by a slight change in the mobile phase composition, saturation time and results are tabulated in the table 6. The method is not altered by changing the method parameters like mobile phase composition, chamber saturation time and scanning wavelength, which proves that the method is robust. The work was applied to estimate DAPA in tablet dosage form, the percentage purity was found to be 100 \pm 0.05 as shown in the table 7. The validated method showed that the drug content separated with no interfering peaks generated by the excipients in the marketed formulation, as shown in the fig. 5. The method is versatile and simple for the analysis DAPA in pure and pharmaceutical formulations. The method was found to obey all the validation parameters as per the ICH guidelines. The method confirms minimal use of the mobile phase with a short run time compared to other reported analytical methods. This validated method can be used for quality control laboratories for the routine quantitative analysis of API and tablets of DAPA.

CONCLUSION

Based on the studies conducted and the results obtained, it can be concluded that the developed HPTLC method is simple, selective, precise, sensitive and accurate. The developed and validated method may be used for the determination of DAPA in both bulk drug and tablet dosage forms. The excipients used in the formulation were also not interfering in the analysis. The method can also minimize the time and cost of analysis as several samples can be analysed simultaneously with a minimum amount of mobile phase. The method does not require expensive chemical and solvents and can be used as an alternative for existing HPLC methods.

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AUTHORS CONTRIBUTIONS

B. V. SUMA-Has carried out the HPTLC analysis and validation and prepared the manuscript DEVESWARAN. R-Has carried out the analytical work, validated and helped in manuscript preparation PREMNATH SHENOY-Has helped in procurement of drug, revision and correction of the manuscript.

CONFLICT OF INTERESTS

The authors declare no competing financial interest

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