Research Article

Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Dapagliflozin Propanediol and Metformin Hydrochloride in Tablet Dosage Form

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ARTICLE INFO

ABSTRACT

Rapid, precise, accurate, specific and simple stability indicating RP-HPLC method was developed for simultaneous estimation of dapagliflozin propanediol (DAPA) and metformin hydrochloride (MET) in its tablet dosage form. The method was performed on a column C_8 Thermoquest, hypersil division of dimension 250×4.60 mm having particle size 5 micron. The mobile phase used in the method was 10 mM ammonium acetate buffer (pH-4), methanol, and acetonitrile in a proportion of 30:65:05, respectively. The drug was subjected to acid and alkali hydrolysis, oxidation, photolysis and heat as stress conditions. The method was validated for specificity, linearity, range, precision, accuracy, robustness, LoD, LoQ and system suitability. The flow rate was maintained at 0.8 mL/min and effluent was monitored at 227 nm. The retention time were observed 5.988 minutes and 4.661 min for DAPA and MET, respectively. The standard curve was found linear over range of 60-140 μg/mL for DAPA and 300-700 μg/mL for MET with a correlation coefficient of 0.9996 for DAPA and 0.9994 for MET. The limit of detection (LoD) of this method was 1.121 μg/mL for DAPA and 6.162 μg/mL for MET. The limit of Quantitation (LoQ) of this method was 3.396 μg/mL for DAPA and 18.674 μg/mL for MET. The percentage recovery was found to be in the range of 98–102% at three different levels of standard addition. The precision (repeatability, intra-day and inter-day) of the method was within the limit (RSD < 2%). Degradation products produced because of stress studies did not interfere with DAPA and MET detection, and the assay can thus be considered stability-indicating. The combination tablet was successfully analyzed using the developed method.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration-hyperglycemia (fasting plasma glucose >7.0 mmol/L, or plasma glucose >11.1 mmol/L, 2 hours after a meal)-caused by insulin deficiency, often combined with insulin resistance. Hyperglycaemia occurs because of uncontrolled hepatic glucose output and reduced glucose uptake by skeletal muscle with reduced glycogen synthesis.[1] Type 2 diabetes, previously referred to as "noninsulin-dependent diabetes" or "adult-onset diabetes," accounts for 90–95% of all diabetes. This form encompasses individuals who have relative (rather than absolute) insulin deficiency and have peripheral insulin resistance.[2] Anti-diabetic drugs are medicines developed to stabilize and control blood glucose levels amongst people with diabetes. Anti-diabetic drugs are commonly used to manage diabetes.[3] Patients with type 2 diabetes (T2D) often require multiple anti-diabetic agents to achieve and maintain glycemic control because of the progressive nature of the disease.[4] A stability indicating method (SIM) is defined as a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from process impurities, excipients and degradation products.[5]

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The Dapagliflozin Fig. 1 is chemically known as (1s)-1, 5-anhydro- 1- C- [4- chloro- 3- [(4-ethoxyphenyl) methyl] phenyl]-D-glucitol. It has a molecular formula of $C_{24}H_{33}ClO_8$ with molecular weight 408.98 g/mol. Dapagliflozin is a selective sodium-glucose Co- Transporter 2 inhibitor (SGLT 2). It acts by reducing the reabsorption of glucose by the kidney, leading to excretion of excess glucose in the urine, thereby improving glycemic control in patients with type 2 diabetes mellitus.[6]

Metformin hydrochloride Fig. 2 is chemically known as 1, 1-dimethylbiguanidine monohydrochloride. It has a molecular formula of $C_4H_{11}N_5$. HCl with molecular weight 165.63 g/mol. It is an anti-diabetic drug from the biguanide class of oral hypoglycaemic agents, given orally in the treatment of non –insulin-dependent diabetes mellitus. Major action of metformin HCl is in increasing glucose transport across the cell membrane in skeletal muscle.[7]

A Survey of literature revealed that few methods have been reported for simultaneous DAPA and MET estimation by UV Spectrophotometric[8] and RP-HPLC methods.[9] Two methods are available in the literature for stability-indicating assay of DAPA and MET in combination by using a non-volatile buffer in mobile phase.[10-11] However, as per our knowledge, the stability-indicating assay method of DAPA and MET combined with a volatile buffer in the mobile phase has not been reported till date. Use of volatile buffer in the mobile phase is necessary for a further study like Liquid Chromatography mass spectrometry (LC-MS). The aim of the present work was to develop an easy, economical, accurate, and specific stability-indicating assay method in a volatile buffer for simultaneous estimation of DAPA and MET in combined pharmaceutical formulation. The developed method was validated as per ICH guidelines.[12]

Materials and Methods

Chemicals and Reagents

Morepen Laboratories Ltd., Baddi gifted pure drug sample of DAPA, and MET was gifted by Harman Finochem Ltd., Mumbai. The gift samples were used as standard without any purification. Oxtramet™ XR tablets (Astra Zeneca
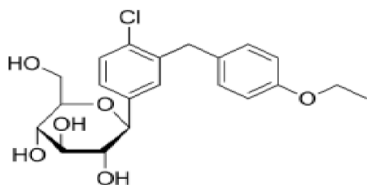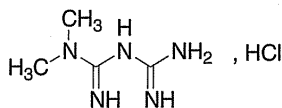

Fig. 1: Dapagliflozin

Fig. 2: Metformin Hydrochloride

Pharmaceuticals LP) containing dapagliflozin propanediol monohydrate equivalent to dapagliflozin (10 mg) and metformin hydrochloride (500 mg) were procured from the local drug market. Methanol (HPLC grade), acetonitrile (HPLC grade), water (HPLC grade), and glacial acetic acid were procured from Finar Ltd. Ammonium acetate and Sodium Hydroxide were procured from Merck Specialities Pvt. Ltd. Hydrochloric acid was procured from Rankem. Hydrogen Peroxide was procured from Neelkanth Pharma.

Instrumentation

A High-performance liquid chromatograph agilent infinity 1260 chromatographic system equipped with 1260 Quat Pump VL and PDA detector was used. Samples were injected at 20 µL. The column C_8 Thermoquest, hypersil division (250*4.60 mm, 5 µm particle size) was used. Data acquisition and integration was performed using EZ Chrom software.

Preparation of Mobile Phase

Accurately weighed 0.77 g of Ammonium Acetate was transferred into a 1000 mL volumetric flask and about 950 mL of water was added. The mixture was sonicated and finally diluted with water up to 1000 mL. Then pH 4 was adjusted with Glacial Acetic Acid. This buffer was filtered through 0.45 micron filter paper to remove particulate matter and mixed with methanol and acetonitrile in the ratio of 30:65:05 into a mobile phase bottle. The prepared mixture was sonicated for 5 minutes in ultrasonic bath for degassing and then used as the mobile phase.

Preparation of Diluent

Based on the drugs' solubility, diluent was selected, Methanol and Water taken in the ratio of 50:50.

Preparation of Standard Stock Solutions

Accurately weighed dapagliflozin propanediol (30.75 mg equivalent to 25 mg dapagliflozin) and metformin Hydrochloride (25 mg) was transferred to individual 25 mL volumetric flask. The drug was dissolved in methanol with sonication and the final volume was adjusted with Methanol up to mark to prepare a 1000 µg/mL stock solution.

Preparation of Standard Working Solutions

From the standard stock solution of DAPA, an accurately measured 1 mL and from the standard stock solution of MET, an accurately measured 5 mL aliquot transferred into 10 mL volumetric flask and final volume was adjusted with diluent up to mark to prepare 100 µg/mL of DAPA and 500 µg/mL of MET respectively.

Preparation of Solutions for Construction of the Calibration Curve

From stock solutions aliquots ranging from 0.6 to 1.4 mL for DAPA and 3–7 mL for MET were taken in 10 mL volumetric flasks respectively, sufficient amount of diluent was added and sonicated, then the volume was made

up to 10 mL with diluent that gave final concentrations 60, 80, 100, 120, 140 µg/mL of DAPA and 300, 400, 500, 600, 700 µg/mL of MET as standard solution of mixtures respectively.

Preparation of Sample Solution

20 tablets were weighed accurately and finely powdered. Powder exactly equivalent to 250 mg of MET and 5 mg DAPA was transferred to a 25 mL volumetric flask. The powder was dissolved in 20 mL of Methanol with sonication for 15 minutes and volume was made up with Methanol. The solution was filtered through Whatman filter paper (No. 42) to get a stock sample solution. An aliquot of 5 mL was pipetted out of a stock sample solution and diluted up to 10 mL with diluent to obtain 100 µg/mL DAPA (Dilution-1: for estimation of DAPA). An aliquot of 1 mL was taken from the above solution and diluted up to 10 mL with diluent to obtain 500 µg/mL MET (Dilution-2: for estimation of MET). These solutions were injected into the chromatographic system to obtain a chromatogram, which was quantified for DAPA and MET.

Method Development and Optimization

The main target of the chromatographic method is to get the separation of closely eluting drugs DAPA and MET. The chromatographic separation was achieved on a stainless-steel column C_8 thermoquest, hypersil division with dimension 250 mm × 4.60 mm having particle size 5 micron. The mobile phase used in the method was 10 mM Ammonium Acetate buffer (pH-4), Methanol and Acetonitrile in a proportion of 30:65:05, respectively. The flow rate of the mobile phase was maintained at 0.8 mL/min. In the optimized conditions, DAPA and MET were well separated with a good resolution and the typical retention times of DAPA and MET were about 5.988 minutes and 4.661 minutes, respectively. The chromatogram of an optimized method for standard drugs individual as well as in binary mixture and for the sample as shown in Figs 3-7. The developed RP-HPLC method was validated.[12]

Force Degradation Study

The forced degradation studies under the conditions of acid, alkali, neutral, hydrogen peroxide, dry heat and sunlight were performed.

Acidic Degradation of Sample

Twenty tablets were weighed accurately and finely powdered. Powder exactly equivalent to 10 mg of DAPA and 500 mg of MET was dissolved in 20 mL Methanol, and 5 mL of 1 N Methanolic HCl was added. Refluxing the solution was carried out heated for 1 hour at 60°C. Then neutralization of the solution was done with 5 mL of 1 N Methanolic NaOH. The resultant solution was diluted up to 50 mL with Methanol. The solution was filtered through Whatman filter paper (No. 42). An aliquot of 5 mL was taken from above solution and diluted up to 10 ml with diluent to obtain 100 µg/mL DAPA (Dilution-1: for

estimation of DAPA). An aliquot of 1 mL was taken from the above solution and diluted up to 10 mL with diluent to obtain 500 µg/mL MET (Dilution-2: for MET estimation) analyzed by HPLC as shown in Figs 8 and 9.

Alkaline Degradation of Sample

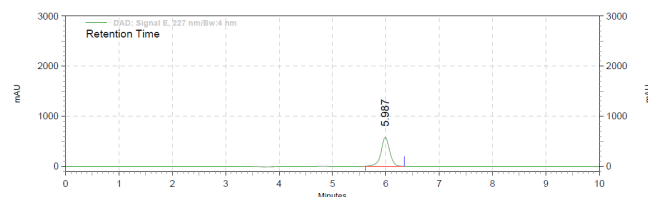Twenty tablets were weighed accurately and finely powdered. Powder exactly equivalent to 10 mg of DAPA
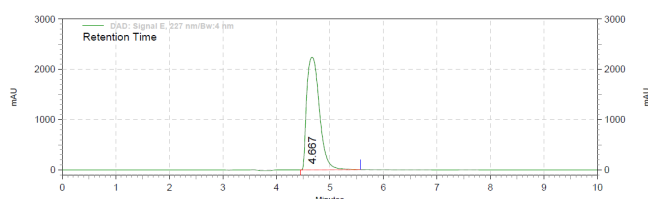

Fig. 3: Chromatogram of DAPA

Fig. 4: Chromatogram of MET
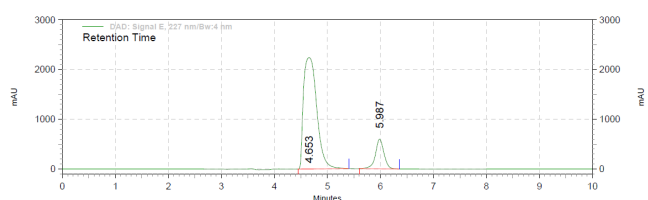

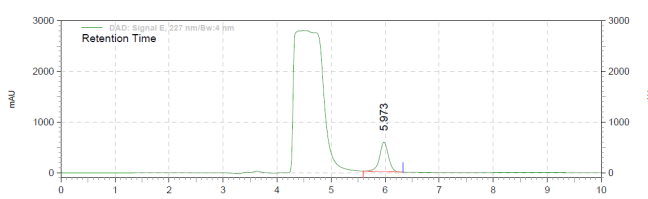**Fig. 5:** Optimized Chromatogram for binary mixture (DAPA -Rt 5.987 min and MET-Rt 4.653 min)

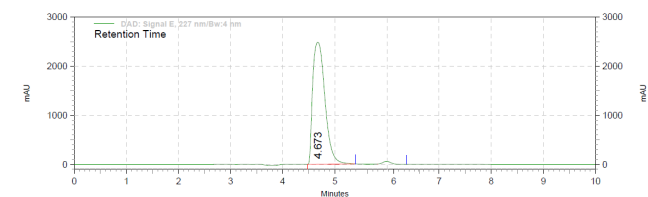**Fig. 6:** Chromatogram of sample for DAPA
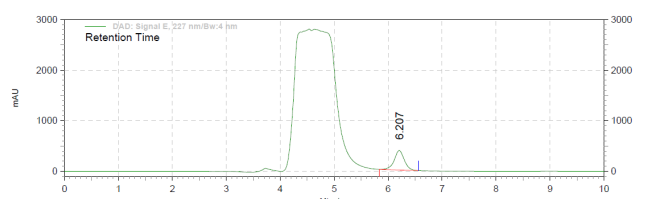

Fig. 7: Chromatogram of sample for MET

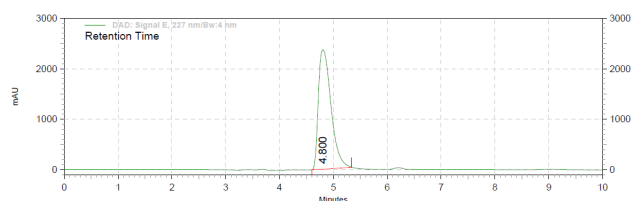**Fig. 8:** Acid degradation in sample for DAPA

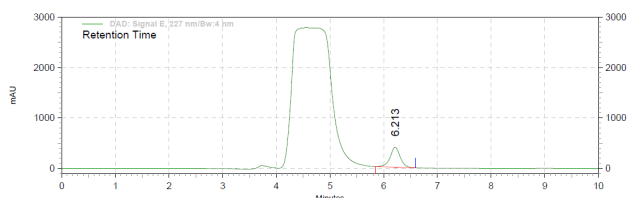**Fig. 9:** Acid degradation in sample for MET

Fig. 10: Alkaline degradation in sample (for DAPA)
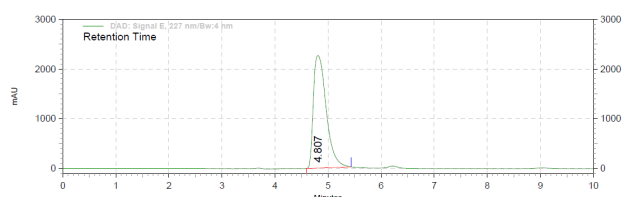

Fig. 11: Alkaline degradation in sample (for MET)
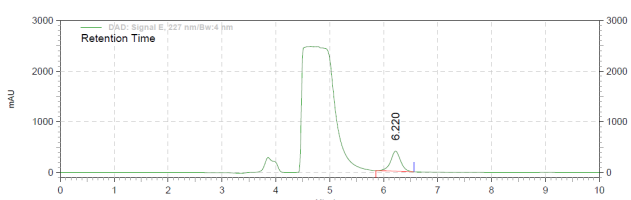

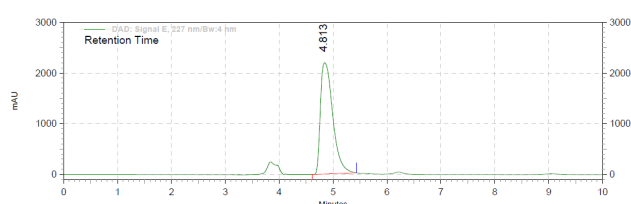**Fig. 12:** Oxidative degradation in sample (for DAPA)

Fig. 13: Oxidative degradation in sample (for MET)

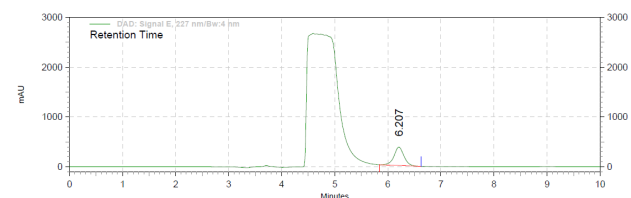**Fig. 14:** Neutral degradation in sample (for DAPA)

Fig. 15: Neutral degradation in sample (for MET)

and 500 mg of MET was dissolved in 20 mL Methanol, and 5 mL of 1 N Methanolic NaOH was added. Refluxing the solution was carried out heated for 1-hour at 60°C. Then neutralization of the solution was done with 5 mL of 1 N Methanolic HCl. The resultant solution was diluted up to 50 mL with Methanol. The solution was filtered through Whatman filter paper (No. 42). An aliquot of 5 mL was taken from above solution and diluted up to 10 mL with diluent to obtain 100 µg/mL DAPA (Dilution-1: for estimation of DAPA). An aliquot of 1 mL was taken from the above solution and diluted up to 10 mL with diluent to obtain 500 µg/mL MET (Dilution-2: for MET estimation) analyzed by HPLC as shown in Figs 10 and 11.

Oxidative degradation of sample

Twenty tablets were weighed accurately and finely powdered. Powder exactly equivalent to 10 mg of DAPA and 500 mg of MET was dissolved in 20 mL Methanol and 5 mL of 6% H_2O_2 was added and kept for 24 hours at Room temperature. The solution was diluted up to 50 ml with Methanol. The solution was filtered through Whatman filter paper (No. 42). An aliquot of 5 mL was taken from above solution and diluted up to 10 mL with diluent to obtain 100 µg/mL DAPA (Dilution-1: for estimation of DAPA). An aliquot of 1 mL was taken from the above solution and diluted up to 10 ml with diluent to obtain 500 µg/mL MET (Dilution-2: for estimation of MET) and analyzed by HPLC as shown in Figs 12 and 13.

Neutral Degradation of Sample

Twenty tablets were weighed accurately and finely powdered. Powder exactly equivalent to 10 mg of DAPA and 500 mg of MET was dissolved in 20 mL Methanol. Refluxing the solution was carried out heated for 1 hour at 60°C. The resultant solution was diluted up to 50 mL with Methanol. The solution was filter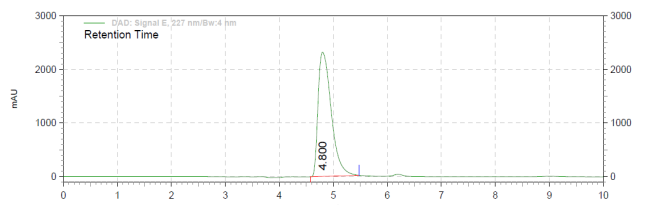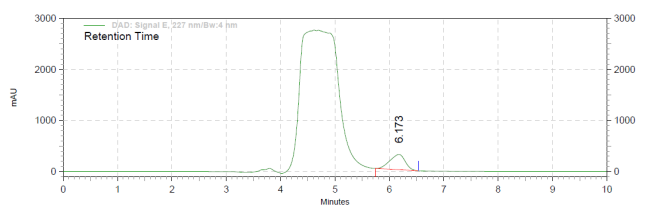ed through Whatman filter paper (No. 42). An aliquot of 5 mL was taken from the above solution and diluted up to 10 mL with diluent to obtain 100 µg/mL DAPA (Dilution-1: for estimation of DAPA). An aliquot of 1 mL was taken from the above solution and diluted up to 10 mL with diluent to obtain 500 µg/mL MET (Dilution-2: for MET estimation) analyzed by HPLC as shown in Figs 14 and 15.

Thermal Degradation of Sample

Twenty tablets were weighed accurately and finely powdered. Powder was kept at 60°C for 8 hours. Powder exactly equivalent to 10 mg of DAPA and 500 mg of MET was dissolved in 20 mL Methanol. The resultant solution was diluted up to 50 mL with Methanol. The solution was filtered through Whatman filter paper (No. 42). An aliquot of 5 mL was taken from above solution and diluted up to 10 mL with diluent to obtain 100 µg/mL DAPA (Dilution-1: for estimation of DAPA). An aliquot of 1 mL was taken from the above solution and diluted up to 10 mL with diluent

Table 1: Results of forced degradation study

Type of degradation	Degradation condition	% Degradation	
		DAPA	MET
Acid degradation	1 N HCl for 1 hour at 60 °C	20.67	0.74
Alkali degradation	1 N NaOH for 1 hour at 60 °C	18.51	0.52
Oxidative degradation	6% H_2O_2 for 24 hours at RT	19.89	0.26
Neutral degradation	1 hour at 60 °C	0.29	0.19
Thermal degradation	8 hours at 60 °C	0.54	0.52
Photolytic degradation	7 days in sunlight	0.60	0.25
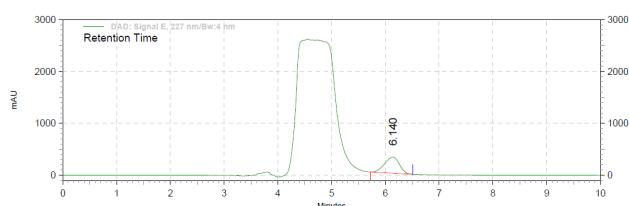
Fig. 16: Thermal degradation in sample (for DAPA)

Fig. 18: Photo degradation in sample (for DAPA)

Fig. 17: Thermal degradation in sample (for MET)

Fig. 19: Photo degradation in sample (for MET)

to obtain 500 µg/mL MET (Dilution-2: for estimation of MET) and analyzed by HPLC as shown in Figs 16 and 17.
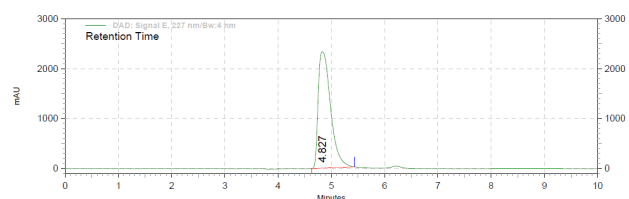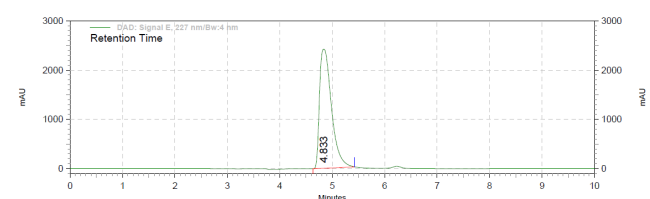
Photo Degradation of Sample

Twenty tablets were weighed accurately and finely powdered. The powder was kept in sunlight for 7 days. Powder exactly equivalent to 10 mg of DAPA and 500 mg of MET was dissolved in 20 mL Methanol. The resultant solution was diluted up to 50 mL with Methanol. The solution was filtered through Whatman filter paper (No. 42). An aliquot of 5 mL was taken from the above solution and diluted up to 10 mL with diluent to obtain 100 µg/mL DAPA (Dilution-1) to estimate DAPA). An aliquot of 1 mL was taken from the above solution and diluted up to 10 ml with diluent to obtain 500 µg/mL MET (Dilution-2: for estimation of MET) and analyzed by HPLC as shown in Figs 18 and 19.

Results and Discussion

Results of Forced Degradation Studies

The results of the forced degradation studies indicated the specificity of the method that has been developed. The DAPA was stable in photolytic, thermal, and neutral stress conditions. Metformin was stable in all stress conditions. The results of forced degradation studies are shown in Table 1.

Results of Method Validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The developed method was validated as per International Council for Harmonisation (ICH) guidelines.[12]

Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of components which may be expected to be present. Method specificity was determined by observing and comparing the test results obtained for sample solution (containing excipients) with standard results obtained for a pure drug. The chromatogram of pure drugs and sample is shown in Figs 3-7.

System Suitability parameters

System suitability parameters were studied to verify the optimum conditions. The system suitability test was performed as per USP guidelines on the chromatograms. Different parameters were evaluated such as retention time, resolution, tailing factor, theoretical plates. The results obtained are summarized in Table 2.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of an analyte in the sample. Minimum five concentrations analyzed for linearity study. A linear response was obtained in

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Table 2: System suitability parameters validated RP-HPLC method

Parameter	Data obtained* ± %RSD	
	DAPA	MET
Retention time (min) ± %RSD	5.988 ± 0.041	4.661 ± 0.099
Theoretical plate per meter ±%RSD	5104 ± 0.553	2704 ± 0.691
Tailing factor ± %RSD	0.953 ± 0.597	1.514 ± 1.705
Resolution ± %RSD	3.848 ± 0.324	-

*Average of six experiments.

Table 3: Recovery from tablet

% SPIKED	Theoretical amount (µg/mL)		Amount found* (µg/mL)		% Recovery ± %RSD	
	DAPA	MET	DAPA	MET	DAPA	MET
80%	80	400	80.52	400.32	100.65 ± 0.746	100.08 ± 0.487
100%	100	500	99.78	501.67	99.78 ± 0.558	100.33 ± 0.504
120%	120	600	120.04	594.73	100.04 ± 0.415	99.12 ± 0.696
Avg.					100.15 ± 0.573	99.85 ± 0.562

*Average of three experiments.

concentrations range of 60–140 µg/mL for DAPA and 300–700 µg/mL for MET. All the solutions were injected, chromatograms were recorded and it was repeated for five times. The linearity graphs were plotted using concentration Vs peak area and a regression equation was derived. The proposed RP-HPLC method showed good linearity with the correlation coefficient, slope, and intercept, 0.9996, 131679, and -16149 for DAPA, 0.9994, 1,64,861.59, and 8,13,582.50 for MET, respectively, as shown in Figs 20 and 21.

Accuracy

The accuracy of the method was determined by recovery experiments using the standard addition method. The 80, 100, and 120% of standard solution were added to a solution of a Tablet. For the two drugs, the recovery studies were performed in triplicate and percentage recovery was calculated. The results found are shown in Table 3.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was determined as Repeatability, Intra-day, and Inter-day. The experiment was repeated 6 times for single concentration at a time for repeatability, three times in a day for intra-day and on 3 different days for inter-day precision for three different concentrations and results were reported as % RSD. The results found are shown in Table 4. From the data obtained, the developed RP-HPLC method was found to be precise.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate
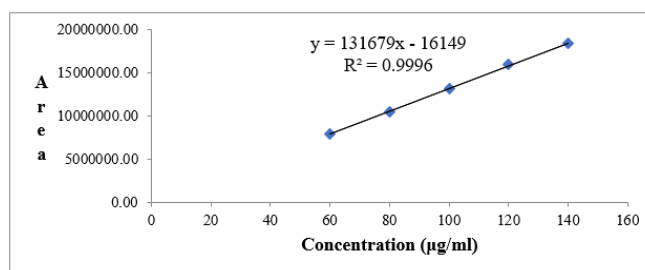
Fig. 20: Calibration curve for DAPA in binary mixture
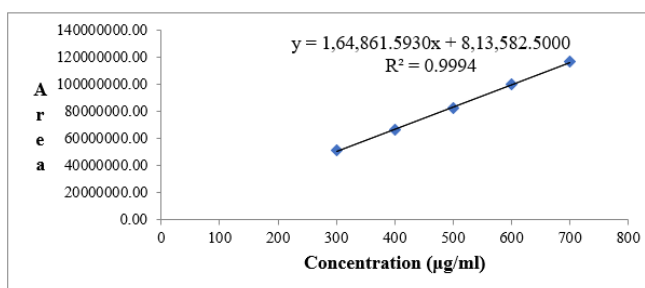
Fig. 21: Calibration curve for MET in binary mixture

variations in method parameters and indicate its reliability during normal usage. Robustness of the method was studied by deliberate variation in method parameters like changes in flow rate, mobile phase ratio, and the mobile phase's pH. It was concluded that method is robust enough and will provide accurate results in normal quality control labs even if there is some sort of experimental error by humans or systems. None of the modifications caused a significant change peak area % RSD. The results found are shown in Table 5.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The LoD is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate

Table 4: Precision for determination of DAPA and MET in binary mixture

Precision	DAPA		MET	
	Conc. (µg/mL)	Area* ± %RSD	Conc. (µg/mL)	Area* ± %RSD
Repeatability	100	13184267 ± 0.467	500	82421916 ± 0.499
Intra-day	60	7875742 ± 0.259	300	50765684 ± 0.401
	100	13122453 ± 0.497	500	82543019 ± 0.390
	140	18335453 ± 0.263	700	116744373 ± 0.364
Inter-day	60	7858411 ± 0.757	300	51011621 ± 0.676
	100	13136759 ± 0.481	500	82391972 ± 0.237
	140	18317715 ± 0.182	700	116041362 ± 0.533

*Average of six experiments for repeatability and three experiments for Intra-day, Inter-day.

Table 5: Results of Robustness

Factor	Level	Area*	
		DAPA	MET
Flow rate	0.7	13402532	84423681
	0.8	13180166	82942101
	0.9	13071570	81999719
%RSD		1.28	1.47
Mobile phase ratio Ammonium Acetate buffer pH 4.0: MeOH: ACN	28:67:05	13279276	82802921
	30:65:05	13180166	82942101
	32:63:05	13234507	82086241
%RSD		0.38	0.56
pH	3.8	13232472	81579276
	4.0	13180166	82942101
	4.2	13173792	81880918
%RSD		0.24	0.87

*Average of three experiments.

Table 6: Assay of Tablet

Oxtramet^TM XR tablets (MET: 500 mg and DAPA: 10 mg)		
	DAPA*	MET*
% ASSAY (mean* ± %RSD)	100.46 ± 1.395	99.65 ± 1.223

*Average of three experiments.

from background levels. The LoQ is defined as the lowest concentration of the standard curve that can be measured with acceptable precision and accuracy. The LoD and LoQ of DAPA and MET were determined by using the slope of the calibration curve and standard deviation (SD) of intercept. The calibration curve was repeated five times and the SD of the intercepts was calculated. Then LoD and LoQ were measured as, LoD = 3.3*SD/slope of calibration curve and LoQ = 10*SD/slope of a calibration curve. The LoD was 1.121 µg/mL for DAPA and 6.162 µg/mL for MET. The LoQ was determined as 3.396 µg/mL for DAPA and 18.674 µg/mL for MET.

Assay of marketed sample

The two drugs combined dosage formulation Oxtramet^TM XR tablets (Astra Zeneca Pharmaceuticals LP) is purchased from local pharmacy and analyzed using the proposed method. For the analysis, three replicates were assayed. The mean peak area of each drug was calculated, and the drug content in the sample was quantified. The results found are shown in Table 6.

References

1. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology. Elsevier. 2007.
2. American Diabetes Association (ADA). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care, 2018; 41(1): S13-S27.
3. https://www.diabetes.co.uk/diabetes-medication/antidiabetic-drugs.html [Last accessed on 17 Aug 2020].
4. Rosenstock J, Hansen L, Zee G, Li Y, Cook W, Hirshberg B and Iqbal N. Dual Add-on Therapy in Type 2 Diabetes Poorly Controlled With Metformin Monotherapy: A Randomized Double-Blind Trial of Saxagliptin Plus Dapagliflozin Addition Versus Single Addition of Saxagliptin or Dapagliflozin to Metformin. Diabetes Care. 2015 Mar; 38(3):376-383.
5. Quadri SS, Sonwane LV, Poul BN and Kamshette SN. Review on Stability Indicating Assay Methods (SIAMs). PharmaTutor. 2014; 2(8):16-31.
6. Eswarudu MM, Babu PS and Aswini R. A Novel RP-HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in Bulk and Pharmaceutical Dosage Form. Int J Pharm Sci Res. 2018; 9(12):5161–5167.
7. Nyola N and Jeyabalan GS. Development and validation of UV-Vis specctroscopy method for simultaneous estimation of Saxagliptin Hydrochloride and Metformin Hydrochloride in active pharmaceutical ingridient. J Pharm Educ Res. 2012; 3(2):19–23.
8. Jani BR, Shah KV and Kapupara PP. Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture. Journal of Bioequivalence Studies. 2015, 1(1):1-8.
9. Urooj A, Sundar PS, Vasanthi R, Raja MA, Dutt KR, Rao KNV and Ramana H. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Dapagliflozin and Metformin in Bulk and in Synthetic Mixture. World J Pharm Pharm Sci. 2017, 6(7): 2139-2150.
10. Abdel-Gawad SA, Al-Tamimi AMS and Adam EH. Chromatographic Simultaneous Quantification of Dapagliflozin and Metformin Hydrochloride in Presence of their Degradation Products. Int J Biol Pharm Allied Sci. 2017 Oct, 6(10): 2007-2021.

11. Deepan T, Rao MB and Dhanaraju MD. Development of Validated Stability Indicating Assay Method for Simultaneous Estimation of Metformin and Dapagliflozin by RP- HPLC. Eur J Appl Sci. 2017; 9(4):189-199.

12. International Conference on Harmonization (1996) Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Validation of analytical procedures: Text and Methodology, ICH Q2 (R1); 2005.