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Research Article

Development and Validation of the Stability indicating Assay Methodology Employing LC-MS/MS for Concurrent Quantification of Dapagliflozin Propanediol Monohydrate and Metformin Hydrochloride: Probable Degradants based on Mass Spectra

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ABSTRACT

For the concurrent measurement of dapagliflozin propanediol monohydrate (DAPA) and metformin hydrochloride (MET) in combined dosage form, a quick, accurate, specific and easy liquid chromatography tandem mass spectrometry (LC-MS/MS) approach was created. The method was performed on a column C_8 RRHD Eclipse (150 × 4.60 mm, 5 µm). In 5 mM ammonium acetate buffer pH-4.0, methanol and acetonitrile were the components of the mobile phase in the ratios of 30:65:05, respectively. The effluent was detected at 227 nm at a 0.4 mL/min flow rate. The observed retention times for DAPA and MET were 7.297 and 3.230 minutes, respectively. The drug was stressed by being exposed to acid and alkali hydrolysis. From the mass spectra, it was found that two degradant peaks were observed in the standard mixture and the sample during alkali stress condition and probable degradants formed. The developed approach was validated in accordance with ICH guidelines. With correlation coefficients of 0.9969 for DAPA and 0.9975 for MET, it was discovered that the standard curve was linear over the range of 60 to 140 and 300 to 700 µg/mL for DAPA and MET, respectively. The limit of detection (LoD) was 2.959 and 8.893 µg/mL for DAPA and MET, respectively. The limit of quantification (LoQ) was 8.967 and 26.949 µg/mL for DAPA and MET, respectively. The %recovery was determined in between 98 to 102%. The precision was within the limit (Relative Standard Deviation (RSD) <2%). The proposed stability indicates that LC-MS/MS method can be successfully utilized for simultaneous estimation of DAPA and MET in combined dosage form without any prior separation of individual drugs and no interference was found due to degradant formed during stress condition.

INTRODUCTION

A chronic metabolic illness called diabetes mellitus causes hyperglycaemia, or elevated blood sugar levels, which is commonly accompanied by insulin resistance. Uncontrolled glucose production by the liver, decreased skeletal muscle glucose absorption , and decreased glycogen synthesis result in hyperglycaemia.[1] For 90 to 95% of instances of diabetes are type 2 diabetes, also referred to as “adult-onset diabetes” or “noninsulin-dependent diabetes”. This group includes those with relative insulin insufficiency and peripheral insulin resistance.[2] Hyperglycaemia is significantly linked to the progression of type 2 diabetes, which increases the trouble of myocardial infarction, stroke, microvascular incidents, and mortality.[3] Hyperglycaemia is significantly linked to the progression of type 2 diabetes, which increases the risk of myocardial infarction, stroke, microvascular events, and mortality.[4] Because type 2 diabetes is a progressive condition, patients frequently need a combination of antidiabetic medications to attain and maintain glycemic control.[5] Keeping blood sugar levels under control seeks to prevent hyperglycemia’s acute osmotic symptoms, to

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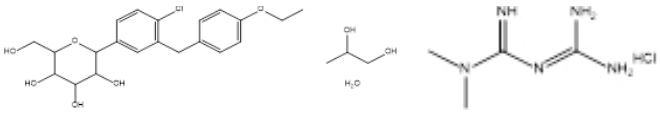


(a) Dapagliflozin Propanediol Monohydrate (b) Metformin Hydrochloride`

Fig. 1: Chemical structure

avoidance of long-term blood glucose instability and delay or prevent the onset of diabetic complications without compromising the quality of life.[6]

Chemically, dapagliflozin is referred to as (1s)-1,5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl)methyl] phenyl]-D-glucitol as illustrated in Fig. 1(a). Its molecular weight is 408.98 g/mol and its chemical formula is $C_{24}H_{33}ClO_8$. Dapagliflozin propanediol monohydrate specifically blocks the sodium-glucose co-transporter 2. It enhances glycemic control in people having type 2 diabetes by stopping kidneys from absorbing again glucose, causing the excess to be discharged in the urine.[7]

Chemically, metformin hydrochloride is referred to as 1,1-dimethylbiguanidine monohydrochloride as illustrated in Fig. 1(b). Its molecular weight is 165.63 g/mol and its chemical formula is $C_4H_{11}N_5$. HCl. It is a biguanide class oral hypoglycemic medication used to treat non-insulin-dependent diabetes mellitus. The main effect of metformin HCl in skeletal muscle is to increase glucose transport across the cell membrane.[7]

A combination of dapagliflozin and metformin is marketed as an Oxtramet™ XR tablet containing 10 mg of dapagliflozin and 500 mg of metformin hydrochloride. A combination of these two drugs is indicated for the treatment of type-2 diabetes.

According to the review of literature, few methods have been published for concurrent determination of DAPA and MET by UV spectrophotometric,[8,9] RP-HPLC method, [10-14] RP-UHPLC method[15] HPTLC[16] LC-MS[17] stability-indicating assay methods[7] and bioanalytical method,[18] LC-MS in human plasma.[19] However, to our knowledge, no reports of the DAPA and MET combined LC-MS/MS approach coupled with mass spectra-based probable degradants have been made as of yet. The objective of the current work was to create a simple, affordable, precise, and stability-indicating test method and determination of probable degradants using LC-MS/MS for concurrent determination of DAPA and MET in the combined dosage form. The established LC-MS/MS technique was validated in accordance with ICH requirements.[20]

Material and Methods

Chemicals and Reagents

Morepen Laboratories Ltd., Baddi supplied a pure medicine sample of the dapagliflozin propanediol monohydrate and Harman Finochem Ltd., Mumbai supplied a pure medicine sample of the metformin hydrochloride. Dapagliflozin Propanediol Monohydrate, which is equivalent to 10 mg of Dapagliflozin and 500 mg of metformin hydrochloride are both found in Oxtramet™ XR tablets (Astra Zeneca Pharmaceuticals LP), which were purchased from local pharmacy. Methanol (LC-MS grade), acetonitrile (LC-MS grade) and ammonium acetate were procured from Sigma-Aldrich, Merck. Sodium hydroxide, hydrochloric acid, glacial acetic acid and hydrogen peroxide were procured from Central Drug House Pvt. Ltd.

Instrumentation

The LC-MS/MS system is equipped with Agilent LC 1290 Infinity 2 chromatographic system, Autosampler (G7129B), Quaternary Pump (G7104A), DAD Detector (G7115A). A Mass 6470 LC/TQ interfaced with the LC via electrospray ionization (ESI) was used. Agilent MassHunter Qual 10.0 software were used for LC-MS/MS data Processing.

Preparation of Mobile Phase

A 1000 mL volumetric flask was filled with precisely weighed 0.38 g of ammonium acetate, and 950 mL of water was then added. The mixture was sonicated and finally diluted up to the mark with water. Then pH 4 was adjusted with glacial acetic acid. This prepared buffer (5 mM) was passed on 0.45-micron filter paper to eliminate contaminants. The buffer was mixed with Methanol and Acetonitrile in a 30:65:05 (% v/v/v) ratio into a mobile phase bottle. The resulting mixture was degassed by sonication in an ultrasonic bath for 5 minutes before it was utilised as a mobile phase.

Preparation of Standard Stock Solutions

Dapagliflozin propanediol monohydrate (30.75 mg, equal to 25 mg DAPA) as well as metformin hydrochloride (25 mg), were precisely weighed and added to individual 25 mL volumetric flasks. Sonication was used to dissolve the medication in methanol and sufficient methanol was added to the final volume to make a 1000 µg/mL stock solution.

Preparation of Solutions for the Development of the Calibration Curve

Aliquots ranging from 0.6 to 1.4 mL and 3 to 7 mL for DAPA and MET, respectively were taken in 10 mL volumetric flasks from standard stock solutions and a sufficient amount of mobile phase was added, then the volume was made up to 10 mL with mobile phase to obtain final concentrations 60, 80, 100, 120 & 140 µg/mL and 300, 400, 500, 600 & 700 µg/mL of DAPA and MET, respectively as the standard solutions of binary mixtures.

Preparation of Sample Solution

Twenty tablets were precisely weighed and finely pulverised. Powder equal to 5 mg of dapagliflozin and 250 mg of metformin hydrochloride was weighed

accurately and transferred in a 25 mL volumetric flask and a sufficient amount of methanol was added to dissolve the drug. The final volume was adjusted with methanol after it was ultrasonically processed for 20 minutes. To get a stock sample solution, the solution was filtered with whatman filter paper (No. 42). An aliquot of 5 mL from a stock sample solution was pipetted out and diluted up to 10 mL with diluent to produce 100 µg/mL DAPA (Dilution-1: for quantification of DAPA). An aliquot of 1-mL of the aforementioned solution was taken, and 10 mL of diluent was added to it to dilute it up to 500 µg/mL MET (Dilution-2: for quantification of MET). These sample solutions were assayed as per the proposed method and the % assay was calculated.

Experimental

Method development and optimization

A stainless-steel column, Agilent Zorbax RRHD Eclipse plus C8, measuring 150 x 4.60 mm and having 5 µm-sized particles was used to develop the chromatographic separation. The mobile phase was composed of 5 mM ammonium acetate buffer pH-4.0, acetonitrile and methanol and it was a mixture of 30:65:05 of each. The mobile phase's flow rate was held constant at 0.4 mL/min. DAPA and MET had good separation and resolution under optimized conditions and their usual retention times were approximately 7.30 and 3.23 minutes, respectively. The chromatography results of a new method for individual standard drugs, binary mixtures, and the sample are displayed in Fig. 2. The developed LC-MS/MS technique was validated.[20]

Forced degradation study of drug substance

Studies on forced degradation were conducted in the presence of acid and alkali conditions.

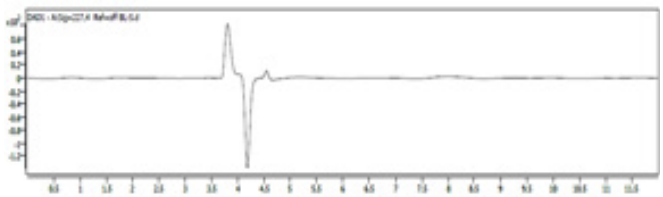
Acidic degradation

- *Acidic degradation of DAPA*

In 50 mg of DAPA was weighed accurately, transfer into a 50 mL volumetric flask and 20 mL Methanol was added to dissolve it. It was added 5 mL of 1 N Methanolic HCl. The resulting solution was refluxed and heated for one hour at 60°C. The solution was then neutralized with 1 N Methanolic NaOH. The resulting solution was diluted with methanol up to 50 mL. The solution was passed through Whatman filter paper (No. 42). An aliquot of 1 mL of the aforementioned solution was taken, and 10 mL of diluent was added to it to dilute it up to 100 μg/mL DAPA. This solution was then analyzed by LC-MS/MS as indicated in Fig. 3(b).

- *Acidic degradation of MET*

In 50 mg of MET was weighed accurately, transfer into a 50 mL volumetric flask and 20 mL methanol was added to dissolve it. It was added 5 mL of 1 N methanolic HCl. The resulting solution was refluxed and heated for one hour at 60°C. The solution was then neutralized with 1 N methanolic NaOH. The resulting solution was diluted with



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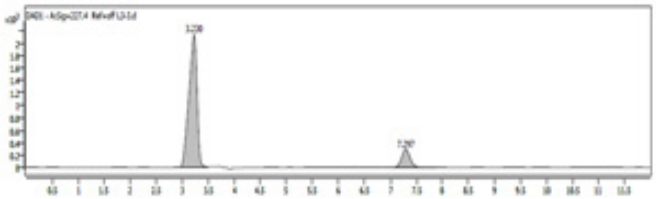
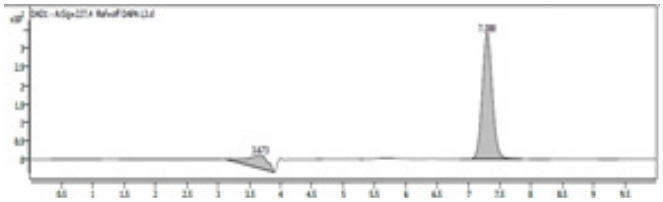
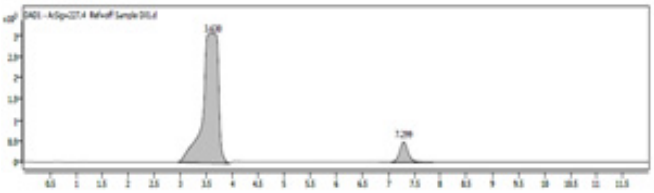


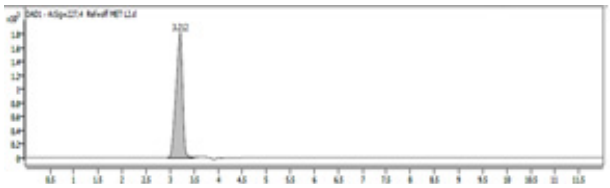
(b) Binary mixture; DAPA (100 µg/mL) -Rt 7.229 minutes and MET (500 µg/mL)-Rt 3.278 minutes



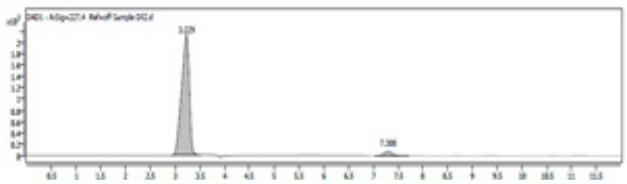
(c) Standard DAPA (100 µg/mL)



(d) Sample for DAPA

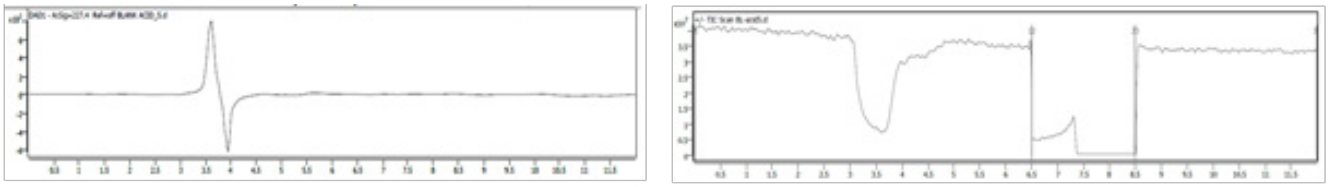


(e) Standard MET (500 µg/mL)



(f) Sample for MET

Fig. 2: Chromatograms for method optimization



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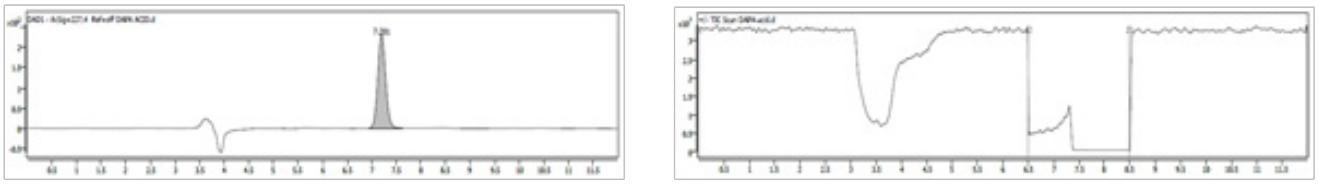
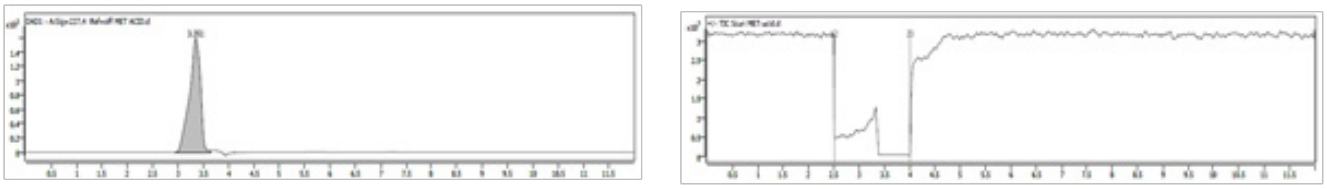
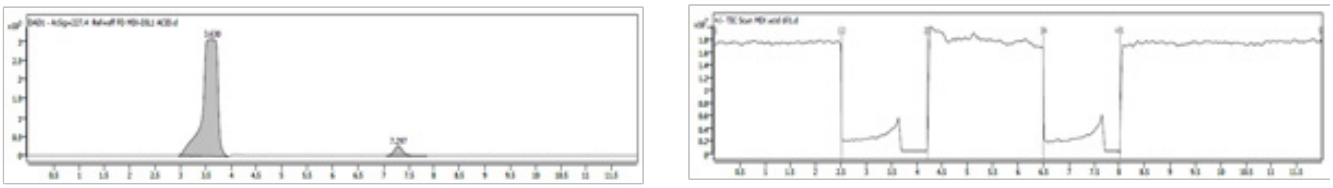


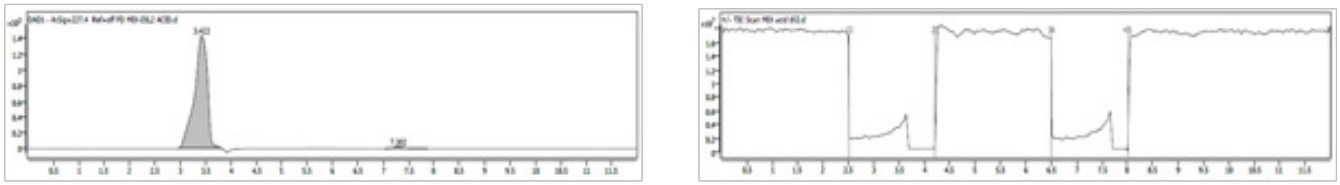
(b) Standard DAPA (100 µg/mL)



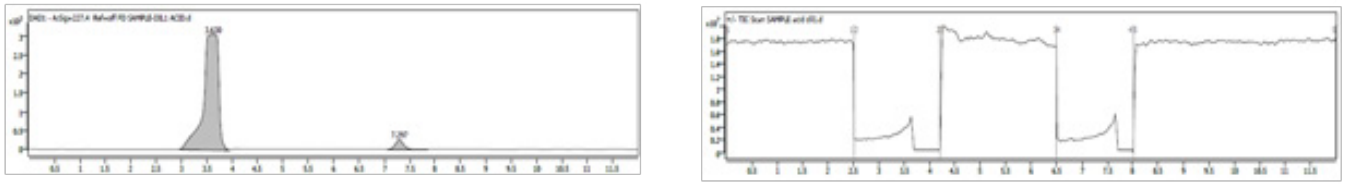
(c) Standard MET (500 µg/mL)



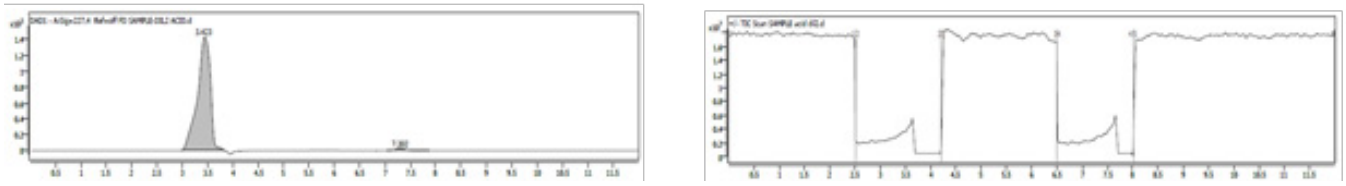
(d) Standard mixture for DAPA



(e) Standard mixture for MET



(f) Sample for DAPA



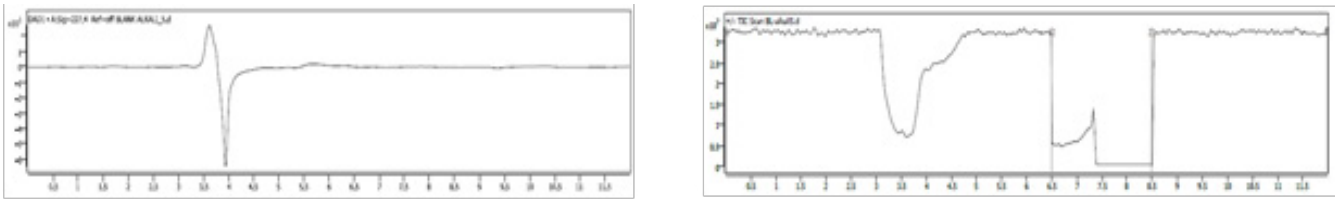
(g) Sample for MET

Fig. 3: Chromatograms and mass spectra for acid degradation

methanol up to 50 mL. The solution was passed through Whatman filter paper (No. 42). An aliquot of 5 mL of the aforementioned solution was taken, and 10 mL of diluent was added to it to dilute it up to 500 μg/mL MET. This solution was then analyzed by LC-MS/MS as indicated in Fig. 3(c).

- *Acidic degradation of DAPA-MET binary mixture*

In 20 mL of methanol was used to dissolve a drug substance precisely equal to 10 mg of DAPA and 500 mg of MET. It was added 5 mL of 1 N methanolic HCl. The resulting solution was refluxed and heated for one hour at 60°C. The solution was then neutralized with 1 N methanolic NaOH. The resulting



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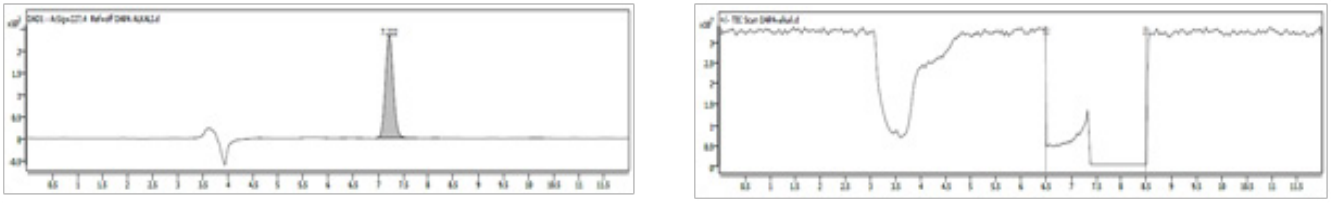
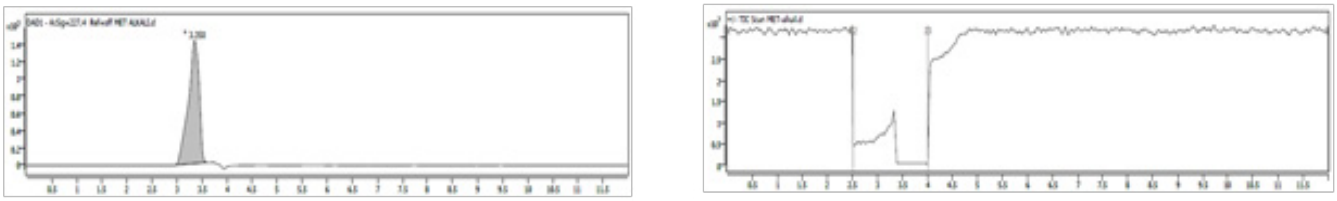
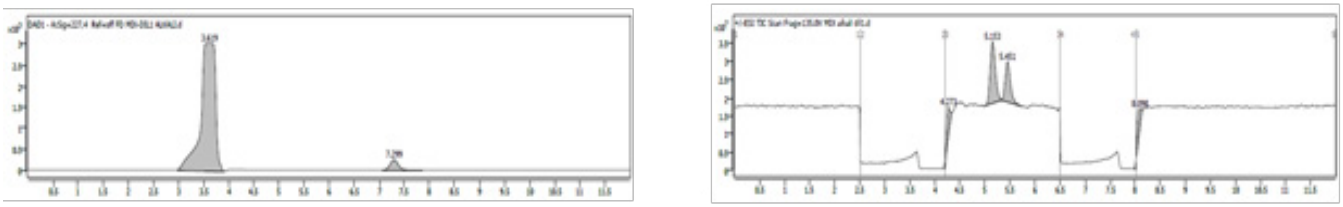


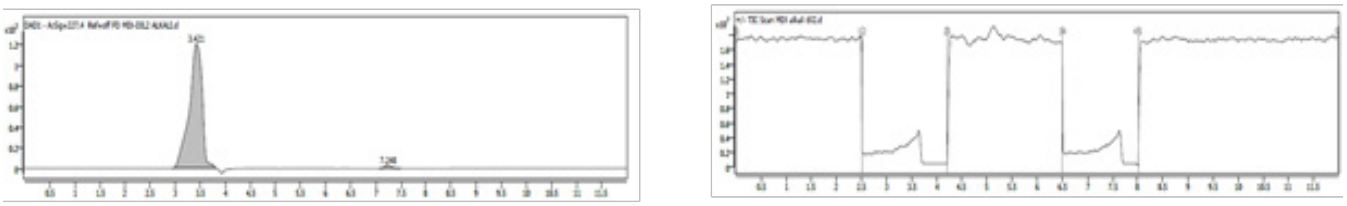
(b) Standard DAPA (100 µg/mL)



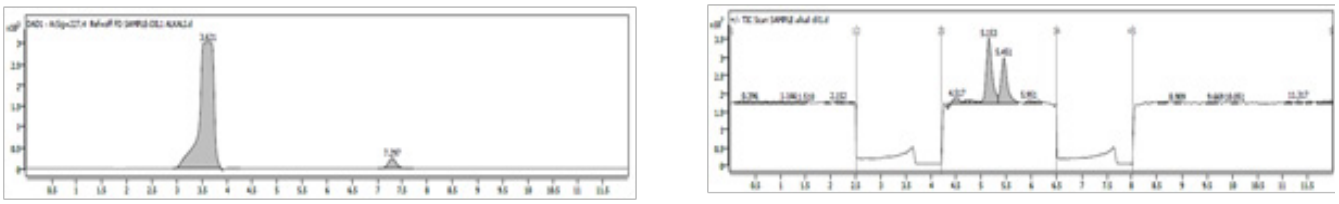
(c) Standard MET (500 µg/mL)



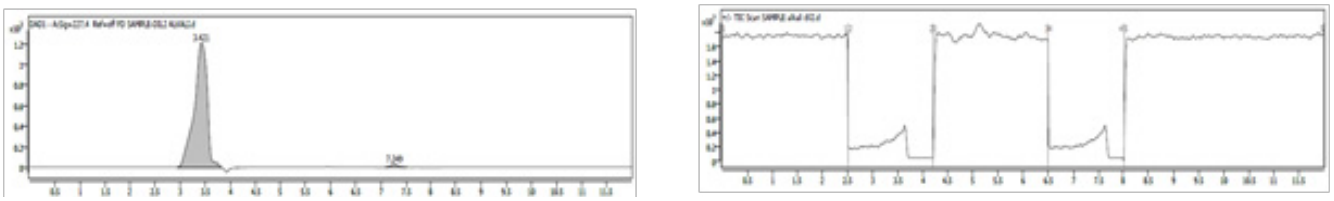
(d) Standard mixture for DAPA



(e) Standard mixture for MET



(f) Sample for DAPA



(g) Sample for MET

Fig. 4: Chromatograms and mass spectra for alkali degradation

solution was diluted with methanol up to 50 mL. The solution was passed through Whatman filter paper (No. 42). To obtain 100 μg/mL DAPA (Dilution-1: for quantification of DAPA), an aliquot of 5 mL from the aforementioned solution was obtained and diluted up to 10 mL with diluent. To obtain 500 μg/mL MET (Dilution-2: for MET quantification), an aliquot of 1-mL from the aforementioned solution was obtained and diluted up to 10 mL with diluent. This mixture was then analysed by LC-MS/MS as indicated in Figs. 3(d, e).

Alkaline degradation

- *Alkaline degradation of DAPA*

In 50 mg of DAPA was weighed accurately, transfer into a 50 mL volumetric flask and 20 mL methanol was added to dissolve it. It was added 5 mL of 1 N methanolic NaOH. The resulting solution was refluxed and heated for one hour at 60°C. The solution was then neutralised with 1 N methanolic HCl. The resultant solution was diluted up to 50 mL with methanol. The solution was passed through whatman filter paper (No. 42). An aliquot of 1-mL of the aforementioned solution was taken, and 10 mL of diluent was added to it to dilute it up to 100 μg/mL DAPA. This solution was then analysed by LC-MS/MS as indicated in Fig. 4(b).

- *Alkaline degradation of MET*

In 50 mg of MET was weighed accurately, transfer into a 50 mL volumetric flask and 20 mL methanol was added to dissolve it. It was added 5 mL of 1 N methanolic NaOH. The resulting solution was refluxed and heated for one hour at 60°C. Then neutralization of the solution was done with 1 N methanolic HCl. The resultant solution was diluted up to 50 mL with methanol. The solution was passed through Whatman filter paper (No. 42). An aliquot of 5 mL of the aforementioned solution was taken, and 10 mL of diluent was added to it to dilute it up to 500 μg/mL MET. This solution was then analyzed by LC-MS/MS as indicated in Fig. 4(c).

- *Alkaline degradation of DAPA- MET binary mixture*

In 20 mL of methanol was used to dissolve a drug substance precisely equal to 10 mg of DAPA and 500 mg of MET. It was added 5 mL of 1 N methanolic NaOH. The resulting solution was refluxed and heated for one hour at 60°C. The solution was then neutralized with 1 N methanolic HCl. The resultant solution was diluted up to 50 mL with methanol. The solution was passed through Whatman filter paper (No. 42). To obtain 100 µg/mL DAPA (Dilution-1: for quantification of DAPA), an aliquot of 5 mL from the aforementioned solution was obtained and diluted up to 10 mL with diluent. To obtain 500 µg/mL MET (Dilution-2: for MET quantification), an aliquot of 1 mL from the aforementioned solution was obtained and diluted up to 10 mL with diluent. This mixture was then analyzed by LC-MS/MS as indicated in Figs. 4d and e.

Forced degradation study of the marketed product

- *Acidic degradation*

Twenty tablets were accurately weighed and finely pulverized. In 20 mL of methanol was used to dissolve a powder precisely equal to 10 mg of DAPA and 500 mg of MET. It was added 5 mL of 1 N Methanolic HCl. The resulting solution was refluxed and heated for one hour at 60°C. The solution was then neutralized with 1 N methanolic NaOH. The resultant solution was diluted up to 50 mL with methanol. The solution was passed through Whatman filter paper (No. 42). To obtain 100 µg/mL DAPA (Dilution-1: for quantification of DAPA), an aliquot of 5 mL from the aforementioned solution was obtained and diluted up to 10 mL with diluent. To obtain 500 μg/mL MET (Dilution-2: for MET quantification), an aliquot of 1-mL from the aforementioned solution was obtained and diluted up to 10 mL with diluent. This mixture was then analysed by LC-MS/MS as indicated in Figs 3f and g.

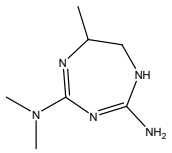
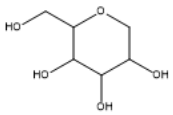
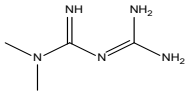
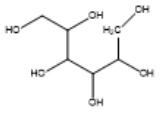
- *Alkaline degradation*

Twenty tablets were accurately weighed and finely pulverized. In 20 mL of methanol was used to dissolve a powder precisely equal to 10 mg of DAPA and 500 mg of MET. It was added 5 mL of 1 N Methanolic NaOH. Refluxing the resultant solution was carried out and heated for 1 hour at 60°C. The solution was then neutralised with 1 N Methanolic HCl. The resultant solution was diluted up to 50 mL with methanol. The solution was passed through whatman filter paper (No. 42). To obtain 100 µg/mL DAPA (Dilution-1: for quantification of DAPA), an aliquot of 5 mL from the aforementioned solution was obtained and diluted up to 10 mL with diluent. To obtain 500 µg/mL MET (Dilution-2: for MET quantification), an aliquot of 1-mL from the aforementioned solution was obtained

Table 1: Results of the forced degradation study

Type of degradation	*Degradation condition*	*Standard mixture/Sample*	*Area*		*%Degradation*	
			DAPA	*MET*	*DAPA (%)*	*MET (%)*
No stress condition	-	Standard mixture	3419	23676	-	-
Acid degradation	1 N HCl for 1 hour at 60°C	Standard mixture	2797	23577	18.19	0.42
		Sample	2802	23541	18.05	0.57
Alkali degradation	1 N NaOH for 1 hour at 60°C	Standard mixture	2759	22102	19.30	6.65
		Sample	2740	22032	19.86	6.94

Table 2: Mass-spectra interpretation (standard mixture and sample) and probable degradants/possible structures

Rt (min)	*Mode*	*m/z ratio*	*Observation*	*Probable degradants*	*Possible structure*
5.153 minutes	+ESI	170.3	It may be due to a reaction between metformin and propendiol from dapagliiflozin propendiol monohydrate	IUPAC Name: N^4,N^4,6-trimethyl-6,7-dihydro-1H-1,3,5-triazepine-2,4-diamine Molecular Weight: 169.23	
	-ESI	162.8	It may be due to the removal of sugar moiety from dapagliflozin	IUPAC Name: 2-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol Molecular Weight: 164.16	
		130.3	It may be due to metformin	Molecular wt. for Metformin-129.16	
5.451 minutes	+ESI	183.3	It may be due to opening the chain for sugar moiety and reaction with sodium hydroxide	IUPAC Name: hexane-1,2,3,4,5,6-hexaol Molecular Weight: 182.17	

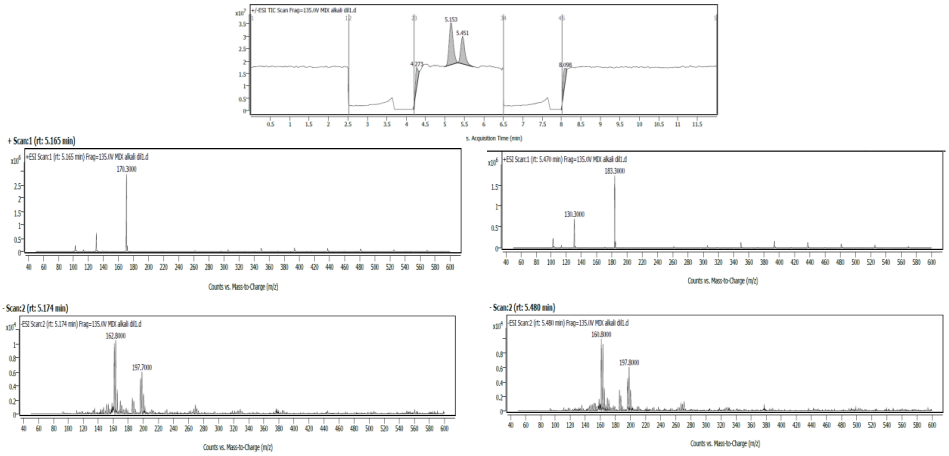


Fig. 5: Mass spectra interpretation- standard mixture

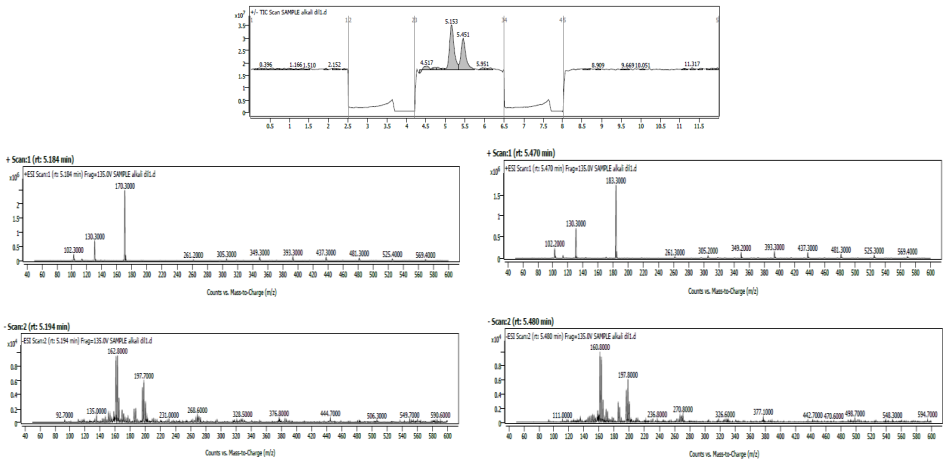


Fig. 6: Mass spectra interpretation- sample

and diluted up to 10 mL with diluent. This the mixture was then analysed by LC-MS/MS as indicated in Figs. 4 (f, g).

Results and Discussion

Results of Forced Degradation Studies

The outcomes of the research on forced deterioration demonstrated the method's specificity. There was >18% degradation of DAPA and >6% degradation of MET in acid and alkali conditions. Table 1 displays the outcomes of studies on forced deterioration. It has been observed from the graphs there is no degradant peak is observed during acidic degradation. It has been observed from the graphs, that there is no degradant peak observed for individual standards of DAPA and MET, but two degradant peaks are observed in mass spectra in the standard mixture and sample during alkali degradation as shown in Fig. 4. Based on the m/z ratio as shown in Figs. 5 and 6, Probable degradants/possible structures are illustrated in Table 2.

Method Validation

Specificity

By comparing the test findings for the sample solution, which contains excipients, with the standard results for a pure medication, the method's specificity was ascertained. Fig. 2 displays the chromatogram of the pure drugs and samples.

System suitability parameters

The ideal circumstances were confirmed by examining the system suitability criteria. In accordance with USP specifications, the chromatograms were used for the system suitability test. The retention times for both drugs were evaluated for SST. Table 3 displays a summary of the outcomes.

Table 3: System suitability parameters validated LC-MS/MS method

Parameter	*Data obtained* ± %RSD*	
	DAPA	*MET*
Retention time (min) ± %RSD	7.288 ± 0.116	3.229 ± 0.061

Linearity and range

For the linearity studies, at least five concentrations must be tested. For DAPA and MET, a linear response was achieved in the concentration range of 60 to 140 µg/mL and 300 to 700 µg/mL, respectively. After injecting each solution, recording the chromatograms, repeating the process five times and the results were analysed. Utilising the concentration vs. peak area plots for the linearity graphs, a regression equation was created. The proposed LC-MS/MS approach showed good linearity, as shown in Fig. 7, with the correlation coefficient, slope, and intercept for DAPA being 0.9969, 36.60 and -104.76, and for MET being 0.9975, 46.49 and 46.4854, respectively.

Accuracy

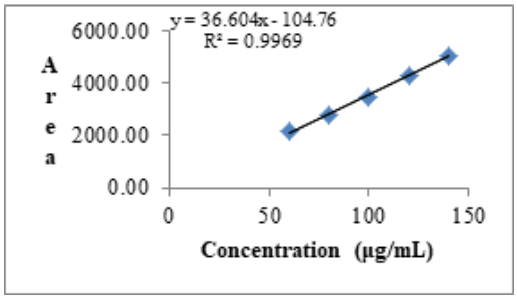
Recovery studies with the traditional addition approach were used to evaluate the method's accuracy. The 80, 100, and 120% of standard solution were added to a solution of a tablet. For the two drugs, the recovery studies were performed in triplicate and percentage recovery was calculated. The outcomes are displayed in Table 4.

Precision

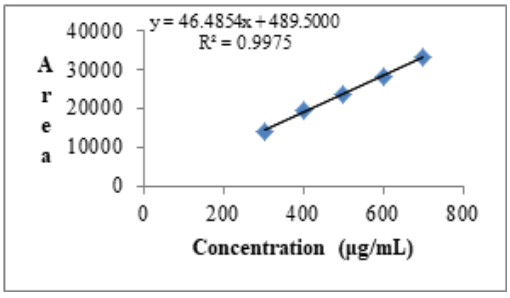
Repeatability, intra-day precision and inter-day precision were used to gauge the method's precision. For repeatability, the experiment was repeated six times for a single concentration at a time, three times within a day and three distinct days for inter-day precision for three different concentrations. Results were presented as %RSD. The results of the experiments are presented in Table 5. The developed LC-MS/MS method was found to be precise based on the data obtained.

Limit of detection and limit of quantitation

The slope from a calibration curve and the SD (standard deviation) of the intercept were used to calculate the limits of detection and quantitation for DAPA and MET. Five times the measurement of the calibration curve was repeated and each time the intercepts' SD was computed. Then, LoD and LoQ were calculated as follows: LoD = 3.3*SD/slope of a calibration curve and LoQ = 10*SD/slope of a calibration curve. For DAPA, the LoD was 2.959 µg/mL, while for MET, it was 8.893 µg/mL. The LoQ was established to be 8.967 µg/mL for DAPA and 26.949 µg/mL for MET.



(a) DAPA



(b) MET

Fig. 7: Calibration curve

Table 4: Recovery study for accuracy (n=3)

% Spiked	Theoretical Amount (µg/mL)		Amount Found (µg/mL)		% Recovery ± %RSD	
	DAPA	MET	DAPA	MET	DAPA	MET
80	80	400	79.06	396.40	98.83 ± 0.453	99.10 ± 1.064
100	100	500	101.29	501.96	101.29 ± 0.281	100.39 ± 0.847
120	120	600	121.38	596.38	101.15 ± 0.991	99.40 ± 0.767
Avg.					100.43 ± 0.575	99.63 ± 0.893

Table 5: Precision for determination of DAPA and MET in binary mixture

Precision	DAPA			MET		
	Conc. (µg/mL)	Area ± SD	%RSD	Conc. (µg/mL)	Area ± SD	%RSD
Repeatability (n = 6)	100	3430 ± 19.54	0.570	500	23746 ± 143.74	0.605
	60	2149 ± 18.72	0.871	300	14091 ± 90.52	0.642
Intra-day (n = 3)	100	3448 ± 49.69	1.441	500	23546 ± 43.02	0.183
	140	5036 ± 43.09	0.856	700	32980 ± 44.84	0.136
	60	2141 ± 15.00	0.701	300	14173 ± 51.05	0.360
Inter-day (n = 3)	100	3439 ± 38.11	1.108	500	23436 ± 221.62	0.946
	140	5062 ± 43.65	0.862	700	33188 ± 172.19	0.519

Table 6: Robustness (n=3)

Factors	Level	Area	
		DAPA	MET
Flow rate	0.35	3511	23720
	0.4	3469	23488
	0.45	3388	22919
	%RSD	1.81	1.76
Mobile phase ratio ammonium acetate buffer pH 4.0: MeOH: ACN	28:67:05	3493	23231
	30:65:05	3469	23488
	32:63:05	3432	23124
	%RSD	0.89	0.80
pH	3.8	3453	23466
	4.0	3469	23488
	4.2	3493	23847
	%RSD	0.59	0.91

Robustness

Intentional modification in the method's parameters, such as changes in flow rate, mobile phase pH and mobile phase ratio was used to examine the method's robustness. It was determined that the technique is reliable enough and would deliver accurate results in typical quality control labs even if there is some kind of experimental error made by people or systems. No alteration changed the peak area %RSD much. Table 6 displays the findings.

Table 7: Assay of Tablet (n=3)

OxtrametTM XR tablets (MET: 500 mg and DAPA: 10 mg)		
	DAPA	MET
% ASSAY (mean ± %RSD)	99.28 ± 1.009	100.37 ± 1.098

Assay of marketed sample

The two drug's combined dosage form (Oxtramet^{TM} XR tablets) is bought from a nearby drugstore and examined using the method that has been recommended. Three replicates of the sample were tested for the analysis. The average peak area of each drug was determined and the amount of each drug in the sample was calculated. In Table 7, the outcomes are displayed.

Conclusion

The proposed stability indicating LC-MS/MS method can be successfully utilized for the simultaneous estimation of DAPA and MET in the combined dosage form without any prior separation of individual drugs and no interference was found due to degradant formed during stress condition. From the mass spectra, it was found that two degradant peaks were observed in the standard mixture and the sample during alkali stress condition and probable degradants formed are discussed earlier. The LC-MS/MS method developed for the analysis of binary mixtures of DAPA and MET is found to be simple, accurate, precise, specific, robust and rapid.

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