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Kinetic Degradation Study of Dapagliflozin Coupled with UHPLC Separation in the Presence of Major Degradation Product and Metformin

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ORIGINAL

Kinetic Degradation Study of Dapagliflozin Coupled with UHPLC Separation in the Presence of Major Degradation Product and Metformin

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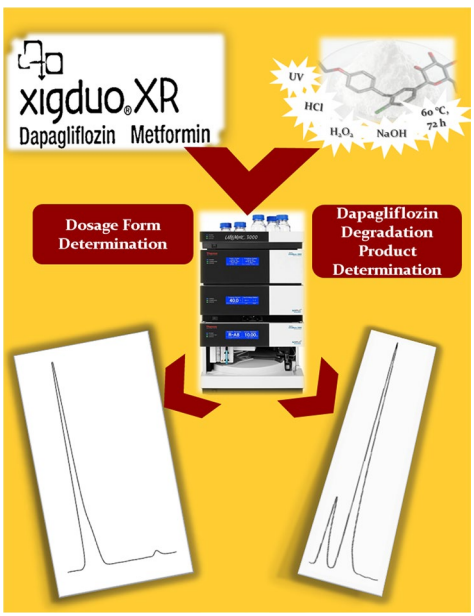
Abstract

A novel ultra-performance liquid chromatography with UV detector technique was established for simultaneous determination of two antidiabetic drugs, dapagliflozin (DAPA) and metformin (MET), followed by a stress degradation study. Main degradation product was chromatographically separated and precisely characterized via LC-MS/MS. Chromatographic separation done on a Symmetry® Acclaim™ RSLC 120 C18 column (100 mm, 2.1 mm, 2.2 µm), column temperature was maintained at 60 °C. Mobile phase was a mixture of potassium dihydrogen phosphate buffer, pH (3.5)—acetonitrile (50:50, *v*/*v*) at flow rate of 0.4 mL/min. The method has displayed an adequate detection at concentration ranges of 1–50 µg/mL for dapagliflozin propanediol monohydrate and 0.5–100 µg/mL for metformin hydrochloride. DAPA was then exposed to different stress conditions include alkaline, acidic, oxidative and ultraviolet light. A study of the degradation kinetics in alkaline medium for DAPA has proved that the degradation follows a pseudo-first-order reaction. The proposed method was effectively applied for the analysis of laboratory prepared mixtures as well as a combined pharmaceutical formulation with 1:200 ratio of DAPA: MET. No significant difference was found regarding accuracy and precision upon statistical comparison between the obtained results and those of the reported method. Validation was conducted in compliance with the ICH guidelines proving that method is selective, linear, precise and accurate. The simplicity and sensitivity of this method allows its use in the quality control tests of the two cited drugs.

Graphical Abstract



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Keywords Dapagliflozin · Metformin · Degradation kinetics · SGLT2 · Xigduo XR · Stability study · HPLC–UV · UHPLC–UV

Introduction

Metformin (MET) and dapagliflozin (DAPA) Fig. 1; the first is best to be described as efficient anti-hyperglycemia legacy agent, comes along with a recently approved and clinically validated medication, dapagliflozin.

Generally metformin is the most accepted first-line treatment in type 2 diabetes mellitus patients. It improves peripheral and hepatic sensitivity to insulin, reduces liver gluconeogenesis and enhances the utilization of glucose by peripheral tissues [1].

DAPA belongs to a new antidiabetic class which is sodium glucose co-transporter 2 (SGLT2) inhibitors. In kidneys, glucose is reabsorbed into the proximal tubule through the sodium-coupled glucose co-transporter (SGLT2). This promotes urinary excretion of glucose and results in lowering of blood glucose level. Administration of agents (e.g., DAPA) that inhibits SGLT2 transporter have shown to be associated with improvement in hyperglycaemia without clinically persistent electrolytes disturbances. This shows why administration of DAPA alone or in combination is effective and safe as a combination treatment for hyperglycaemia [2].

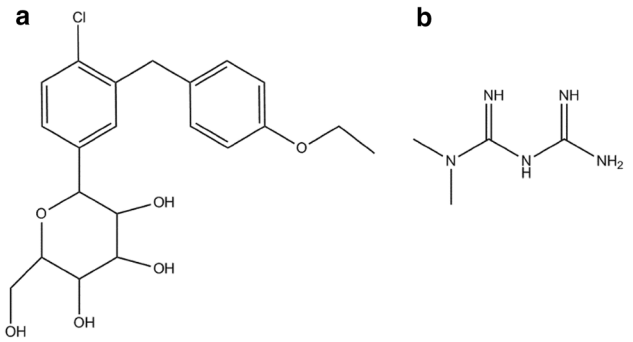


Fig. 1 Dapagliflozin (**a**) and Metformin (**b**) structures

Referring to the literature review; sparse methods were proclaimed [3–7] for concentration assurance of DAPA and MET together in pharmaceutical preparations or in laboratory prepared mixtures. Other few methods were found for determination of DAPA alone, either in dosage form [8–12] or in rat plasma [13]. On the contrary, various analysis methodologies were developed for investigation of MET using both spectrophotometry and chromatographic techniques with many degradation and kinetic studies [13–34]. Owing to the many reported methods of metformin degradation, the scope of the presented work was out of this part.

This work aimed to achieve the first UHPLC method for concurrent separation and concentration quantification of DAPA and MET in laboratory prepared mixtures and in pharmaceutical dosage form with very low ratio 1:200. This is done through spiking and standard addition technique followed by applying the chromatographic method. It is worth to mention the added value of this proposed study over the previous work on this combination. This is the only UHPLC–UV method for simultaneous determination of DAPA and MET in one chromatogram via applying

Table 1 Comparison between the chromatographic conditions used for separation of metformin and dapagliflozin in different reported HPLC–UV methods and the proposed method

Method	Column	Mobile phase	Flow rate	Linearity range
Proposed method	Symmetry® Acclaim™ RSLC 120 C18 column (100 mm × 2.1 mm, 2.2 µm)	Phosphate buffer: acetonitrile (pH 3.5) (50:50, v/v)	0.4 ml/min	0.5 µg/mL to 100 µg/mL for metformin HCL 1 µg/mL to 50 µg/mL for dapagliflozin
Yunoos et al. [5]	Hypersil BDS-C18 (250 mm × 4.6 mm, 5 µm)	Trimethylamine :acetonitrile (ph 6.8) (50:50, v/v)	1 ml/min	85 µg/mL to 510 µg/mL for metformin HCL 0.5 µg/mL to 3 µg/mL for dapagliflozin
Shyamala. et al. [6] Method	Hypersil BDS-C18 (250 × 4.6 mm, 5 µm)	Phosphate buffer: methanol: acetonitrile (pH 6.5) (50:30:20 v/v/v)	1.0 ml/min	85 µg/mL to 510 µg/mL for metformin HCL 0.5 µg/mL to 3 µg/mL for dapagliflozin
Deepan et al. [7] Method	Inspire-C18 (4.6 mm × 150 mm, 5 µm)	Phosphate buffer: acetonitrile (pH 3.0) (30:70, v/v)	1.0 ml/min	500–2500 µg/mL metformin HCL 5–25 µg/mL dapagliflozin

Table 2 Regression and validation parameters for the determination of the dapagliflozin and metformin by the proposed method

Parameters	DAPA	MET
Linearity range	1–50 μg mL^{-1}	0.5–100 μg mL^{-1}
Slope	1.5216	1.3515
Intercept	0.3729	0.8154
Correlation coefficient	0.9999	0.9999
Accuracy (mean ± SD)	99.72 ± 0.94	99.79 ± 0.51
LOD	1.01 μg mL^{-1}	0.93 μg mL^{-1}
LOQ	3.08 μg mL^{-1}	2.83 μg mL^{-1}
Precision (%RSD) Repeatability	0.16	1.5
Intermediate precision	1.77	1.26
Specificity (mean ± SD)	99.81 ± 1.16	100.46 ± 1.54

spiking technique. In addition, this UHPLC study was done using the least amount of phosphate solution besides the least flow rate ever used which was 0.4 mL/min. The advantages are shown in Tables 1 and 2 which present the pervious methods found in the literature in comparison with the proposed method. Moreover, this piece of work is presenting stress degradation and a kinetic study for DAPA as up to our knowledge; there is no reported kinetic study for its degradation.

Aubry, Gu, Magnier et al. [13] have performed HPLC method for detection of DAPA in plasma using LC–MS/MS in rat plasma. They achieve lowest reported limit of detection and quantification, yet mass detector is considered quiet expensive and less notorious; it may not be used as regular method in quality control laboratories. Where's Aubry, Gu, Magnier et al. aimed to achieve detection in rat plasma that in turn need a higher level of sensitivity, while this UHPLC–UV was driven to perform regular quality supervision of drug in raw or in tablet dosage form. Not to mention that analytical methods utilizing UV detectors are the most widely used in analytical chemistry laboratories.

In general, an UHPLC technique is more preferable than ordinary HPLC method. Budget wise, an UHPLC method is much more convenient as it is more fiscal, it consumes less mobile phase over shorter periods. Furthermore, upon comparison with other reported methods, this proposed method reveals major advantages in terms of shorter retention times, superior sensitivity and boosted resolution.

Experimental

Instruments

The wielded Liquid chromatography facility was a Thermo Fisher UPLC. Its model is Ultimate 3000—Complete Ultra Performance Liquid Chromatography (USA). Separation was done on Symmetry® Acclaim™ C18 column with dimensions (100 mm, 2.1 mm, 2.2 μm) (USA). Diode Array UV Detector (DAD-3000 RS, USA) and an autosampler (WPS-3000TRS, Thermo scientific USA) were employed. Solvent degassing was utilized using Elmasonic S 60 H water bath sonicator (Germany). To adjust pH, Jenway digital pH meter, UK was used.

Chemicals and Reagents

High grades of DAPA propanediol monohydrate powder guaranteed to contain 99.90% of pure raw material drug were purchased from BaoJi Guokang Bio-technology co., Ltd. Metformin hydrochloride, certified to have 99.88% of the active material of the drug, was kindly granted from 'Chemical Industries Development' (CID) Co. (Cairo, Egypt). Xigduo XR® tablet contains 1000 mg MET and 5 mg DAPA was obtained from USA market. HPLC grade methanol, acetonitrile and orthophosphoric acid (85%) were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). Ultrapure grade of potassium phosphate monobasic was bought from Sigma-Aldrich (Deisenhofen, Germany). Deionized and distilled water were provided in house. Mobile phase was filtered through PTFE membrane filters (UK) with diameter 47 mm and pore size of 0.20 mm. Chemicals of analytical laboratory grade were used unless marked differently.

Chromatographic Conditions

Separation was carried out using a Symmetry® Acclaim™ C18 column (100 mm, 2.1 mm, 2.2 μm) via isocratic elution. Potassium phosphate monobasic as buffer (0.05 M) with pH (3.5) and acetonitrile in (50:50, v/v) ratio was prepared as mobile phase. Wavelength of 225 nm was elected for determination of both DAPA and MET. After preparation of buffer, it was filtered using a 0.2-mm membrane filter then degassing for 20 min. The pump was adjusted to deliver mobile phase with 0.4 mL/min flow rate. Temperature of column oven was maintained at 60 °C. Autosampler was set to inject 10 μl of each sample each time. Typical separation chromatogram is represented in Fig. 2.

Procedures

Preparation of Calibration Standards

Stock solutions of DAPA and MET with concentration of 1 mg/mL were solely prepared in methanol. Then working solutions were obtained by dilution of the analogous stock solutions with mobile phase. Both working solutions and

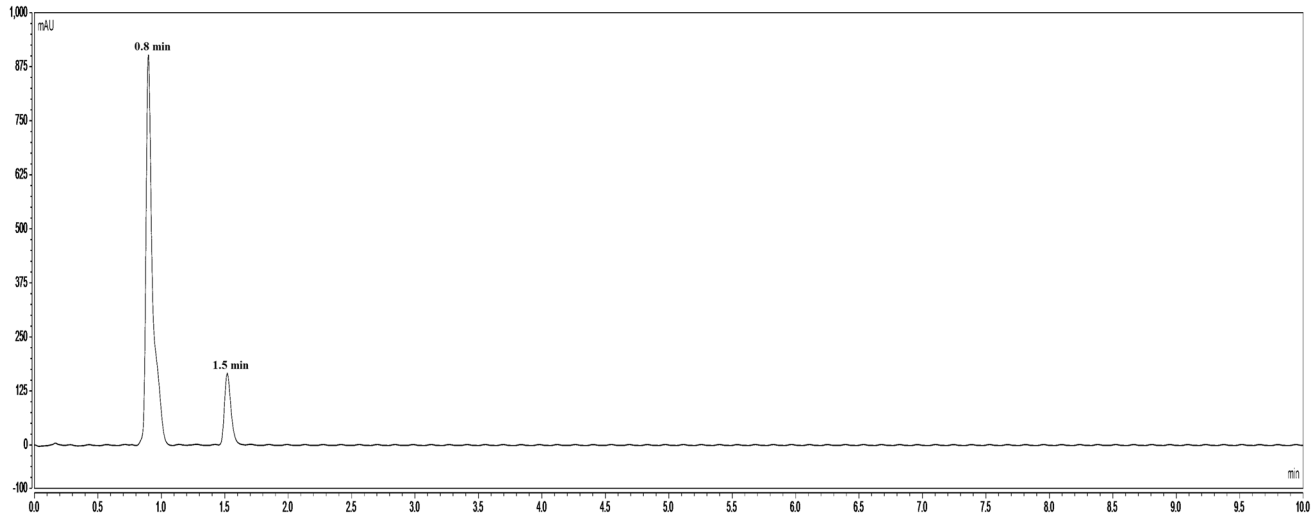


Fig. 2 Chromatogram of laboratory prepared mixture of 60 µg of MET and 10 µg of DAPA under the mentioned conditions "Method Development"

initial stock solutions were stored at 4 °C in refrigerator and shed within 30 days.

Calibration Curves Development

Aliquots between 1 and 50 µg for DAPA and 0.5–100 for µg MET were separately and precisely taken from their working solutions into 10 mL volumetric flasks. Volumes were completed to the final mark using mobile phase; potassium phosphate monobasic: acetonitrile (50:50, v/v) pH 3.5. Final ranges of concentration were: 1–50 µg/mL for DAPA and 0.5–100 µg/mL for MET. 10 µL of each concentration was injected utilizing the mentioned conditions into the chromatographic system. Each injection is done in triplicates. Plotting the value of area under the peak obtained for each sample versus its comparable concentrations, calibration curve was obtained for each drug. Consequently, regression equations were computed.

Assay of Laboratory Prepared Mixtures

To prepare binary mixtures with different ratios, accurately calculated volumes of DAPA and MET working solutions were properly mixed. 10 µL of each mixture were injected onto stationary phase. Percentage recoveries were then estimated using the corresponding regression equations.

Application to Pharmaceutical Preparation

Ten tablets of Xigduo XR® 5 mg DAPA/1000 mg MET were solely weighed, powdered and thoroughly blended. Specific amount of the obtained powder contains 100 mg of MET and 0.5 mg of DAPA were dissolved in 30 mL of methanol in 100 mL volumetric flask, sonicated for 20 min. The solution was filtered and made up to mark with mobile phase. This step accompanied with spiking of precise calculated amount of DAPA pure drug to achieve standard addition technique. Standard addition technique is critical in this experiment, as concentration ratio in dosage form is 1:200 DAPA to MET which is out of the calibration that was made earlier for DAPA. Hence, calculated pure amount was added to each sample of DAPA, to guarantee its precise overall determination. First, analysis of 0.5:100, DAPA:MET out of dosage form flask was carried out for MET determination, thereafter a known amount of pure standard of DAPA was added making the final concentration within calibration range. Finally, aliquots of 10 µl were injected on C18 stationary phase. Precise calculation of the different concentrations was done via the corresponding regression equations as mentioned under "Calibration Curves Development". Data of sample enrichment and standard addition technique showed in Tables 3 and 4. Figure 3 represents a chromatogram of tablet extract, 100 µg of MET and 5.5 µg DAPA (0.5 µg of DAPA tablet enriched with 5 µg of pure DAPA).

Stress Degradation Conditions of DAPA

Forced degradation studies of bulk drug included appropriate solid state and solution state were done in accordance with the ICH regulatory guidance [36]. Prior to injection, samples were withdrawn at appropriate time, neutralized (in case of acid and alkali hydrolysis) and the solutions were diluted with acetonitrile or mobile phase.

Table 3 Statistical comparison between the proposed UHPLC method and the reported one for the analysis of dapagliflozin [5] and metformin [28] in pure form

Item	Dapagliflozin		Metformin	
	Proposed	Reported	Proposed	Reported
Mean* ± SD	99.72 ± 0.94	100.04 ± 0.45	99.79 ± 0.51	100.59 ± 0.81
% RSD	0.94	0.45	0.51	0.81
n	5	3	5	6
Variance	0.88	0.20	0.26	0.66
t test**(2.262)	0.5408		2.097	
F test (6.2561)	4.363		2.522	

SD standard deviation, *% RSD* percent relative standard deviation

**Values in parenthesis are the theoretical values of *t* and *F* at $P = 0.05$ [35]

For DAPA alkaline and acidic hydrolysis, accurately weighed amount of 5 mg of drug was treated separately in a 25-mL volumetric flask with certain volumes of 0.1 M, 0.5 M and 1 M sodium hydroxide to make final concentration of 200 µg/mL. The same was done for acidic conditions with 0.1 M, 0.5 M and 1 M hydrochloric acid. The solutions were heated in thermostatic water bath at 70 °C for successive time intervals 5, 10, 15, 20, 30, 60 and 120 min and then 1 mL from each solution was taken into 10 mL volumetric flask and neutralized with pre-calculated volumes of acid and base (hydrochloric acid and sodium hydroxide), respectively. Then complete to final volume with mobile phase for acidic conditions and with acetonitrile in case of basic degradation to prevent salting out of the phosphate buffer used in mobile phase. Final concentrations of 20 µg/mL of DAPA were obtained in each flask.

For thermal degradation, an accurately weighed amount of 5 mg of DAPA solid powder was treated and heated for 72 h at 60 °C followed by cooling, dissolution and further dilution to 20 µg/mL. Then it was injected on the stationary phase.

For oxidative degradation, an accurately weighed amount of 5 mg of DAPA treated separately with 6% and 30% (v/v) hydrogen peroxide to reach final concentrations of 3% and 15% (v/v) after dilution, respectively. The prepared mixtures were kept at room temperature for 12 and 5 h, respectively. Thereafter, 10 microliters of the resultant solutions were injected onto column and the chromatograms were run as described before.

The photochemical degradation of the drug was also studied by exposing the dry powder to UV light of 8 W power for 24 h. Powder was then dissolved and diluted with mobile phase. Then 10 microliters of the resultant solutions were injected onto column and the chromatograms were run as described before. Similar to acidic and basic degradation, the final concentration was elected via dilution with mobile phase to reach optimum concentration of 20 µg/mL before applying the chromatographic separations.

Method Validation

The validation of the proposed method has included linearity, accuracy, precision, and specificity, limits of detection and quantification and robustness according to the procedures recommended by ICH Q2 (R1) [34].

Results and Discussion

The main aim of this work was to establish and validate UHPLC–UV method for the simultaneous estimation of DAPA and MET, accompanied with a stability monitoring degradation study for DAPA under different stress conditions followed by kinetic study. Concerning the stability indicating study; the current International Conference on Harmonization (ICH) guidelines necessitate that stability indicating assay methods should be performed and validated over applying stress testing on the drug under different conditions, including hydrolysis at various pHs, oxidation and thermal degradation [36].

Method Development

During the optimization cycle, several chromatographic conditions were attempted using C18 column (100 mm, 2.1 mm, 2.2 µm) Symmetry® Acclaim™ RSLC 120. Various mobile phase compositions such as methanol with water, or acetonitrile with water, in different proportions were tried in an isocratic mode. Then buffer was tried in different molarities and ratios with either of methanol or acetonitrile. Initially, 0.03 M phosphate buffer used yet it gave no separation, so higher molarity of 0.05 M was then considered and implemented. Acetonitrile accompanied with phosphate buffer of 0.05 M in (50:50, *v*/*v*) at a flow rate of 0.4 mL/min gave sufficient separation and good resolution in isocratic mode between the two drugs. In addition, it gave an optimum resolution of the two peaks of DAPA and its main degradation product. Photodiode array detector (PDA) was set at 225 nm

Table 4 Determination of dapagliflozin and metformin in laboratory prepared mixtures and in Xigdue XR tablets (application of standard addition technique) by the proposed method

Item	Laboratory prepared mixtures								
	Concentration (μg mL^{-1})				Recovery				
	DAPA		MET		DAPA			MET	
	5.0		60.0		98.47			101.65	
	2.5		5.0		100.26			101.76	
	25.0		50.0		98.89			101.26	
	10.0		100.0		100.76			99.19	
	20.0		80.0		100.68			98.43	
Mean* ± SD					99.81 ± 1.16			100.46 ± 1.54	
% RSD					1.16			1.54	
Variance					1.35			2.37	
Item	Xigdue XR								
	Taken concentration (μg. mL^{-1})		Added DAPA		Spiked DAPA		Tablet recovery% after subtraction	MET	
	DAPA (1:200)**	MET	Conc. (μg.mL^{-1})	Recovery%	Conc. (μg.mL^{-1})	Recovery%		Taken conc (μg.mL^{-1})	Recovery%
	0.5	100	5.0	99.94	5.50	100.03	100.93	100.0	99.45
			4.0	100.44	4.50	100.07	98.16	90.0	98.51
			7.0	100.09	7.50	100.01	98.82	80.0	101.19
			10.0	100.31	10.50	100.10	98.46	75.0	100.72
			20.0	100.54	20.50	100.48	98.08	50.0	99.73
Mean* ± SD							98.89		99.92
% RSD							1.18		1.06
Variance							1.39		1.12

% RSD percent relative standard deviation, *SD* standard deviation

*Average of three different determinations

**Ratio present in Xigdue XR® tablets

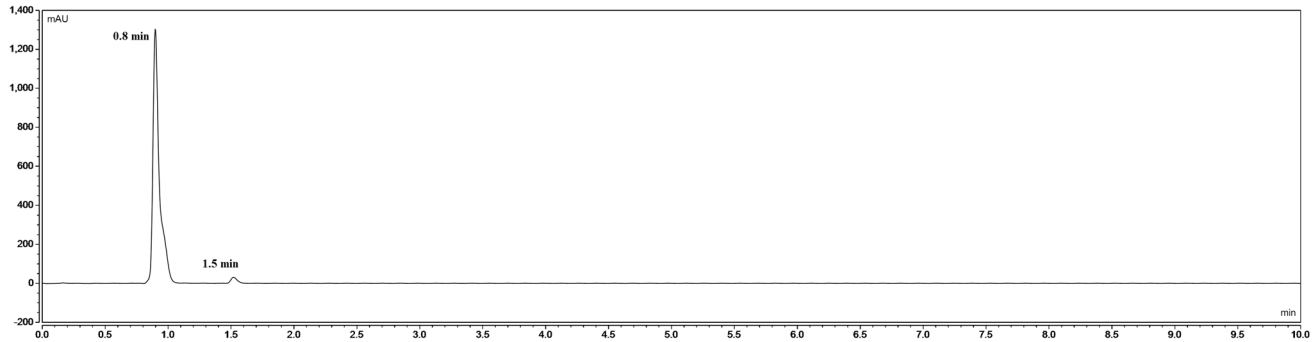


Fig. 3 Chromatogram of tablet extract of Xigdue XR® 100 µg of MET and 5.5 µg DAPA under the mentioned conditions "Method Development" (0.5 µg of DAPA tablet enriched with 5 µg of pure DAPA)

to obtain sufficient peak intensity for both two drugs and DAPA degradation product. A typical chromatogram with sufficient resolution is obtained; MET retention time was at 0.9 min while DAPA was obtained at 1.5 min. The early elution of metformin was due to its polar structure in comparison to dapagliflozin over the reversed phase stationary phase (C18 column).

In sum, our method is efficient and simple to use, if compared with the previously reported methods. Moreover, this is the only method utilizing UHPLC–UV for simultaneous determination of DAPA and MET either in bulk or dosage form.

In addition, stability study for DAPA was carried out under diverse stress conditions (implementing ICH guidelines and optimized conditions from the literature) of heat, UV light, oxidation with hydrogen peroxide and under different pH ranges from highly acidic to highly basic. The presently developed method was also applied to the simultaneous separation of the main degradation product of DAPA and DAPA itself.

The alkaline degradation study, which the kinetic study was also applied, was the most effective one on the drug substance of DAPA. The kinetics of the degradation reaction was carefully monitored with different molarities of sodium hydroxide and various time intervals at constant temperature of 70 °C degrees. The main degradation product was then characterized by UPLC–MS/MS followed by a hypothesis of the expected alkaline degradation product of DAPA.

Many advantages originate from the implementation of UHPLC in this technique. The first advantage is the shorter run time with good chromatogram resolution; this is due to smaller column size. Flow rate used was 0.4 mL/min which is the lowest flow rate used that results in the least solvent consumption, therefore this method is efficient and cost effective to be used in quality control laboratories.

Method Validation

Linearity

Under the optimum chromatographic conditions, DAPA and MET were evaluated by analyzing six different concentrations of each drug in triplicates. Linear relationship between the peak area ratios of each analyte and their corresponding concentrations was established. The linearity ranges were found to be 1–50 µg/mL for DAPA and 0.5–100 µg/mL for MET, results of each calibration represented in Table 2.

Accuracy

The accuracy of the proposed method was assessed by examining five levels of standard solutions of the studied analytes, each three times. The proposed method results were satisfactorily compared with those of reported method for MET [28] and DAPA [5], the results obtained from the statistical analysis showed no significant difference between the performances of the proposed and reference method using Student's *t* test and variance ratio F-test, results showed in Table 2.

Precision

The intraday precision was estimated through triplicate analysis of three different concentrations of the analytes within the same day. On the other hand, the interday precision was achieved through analysis of three different concentrations of the analytes on three successive days. The RSD values were less than 2% proving that the method was precise. The percentage relative standard deviations were calculated as abridged in Table 2.

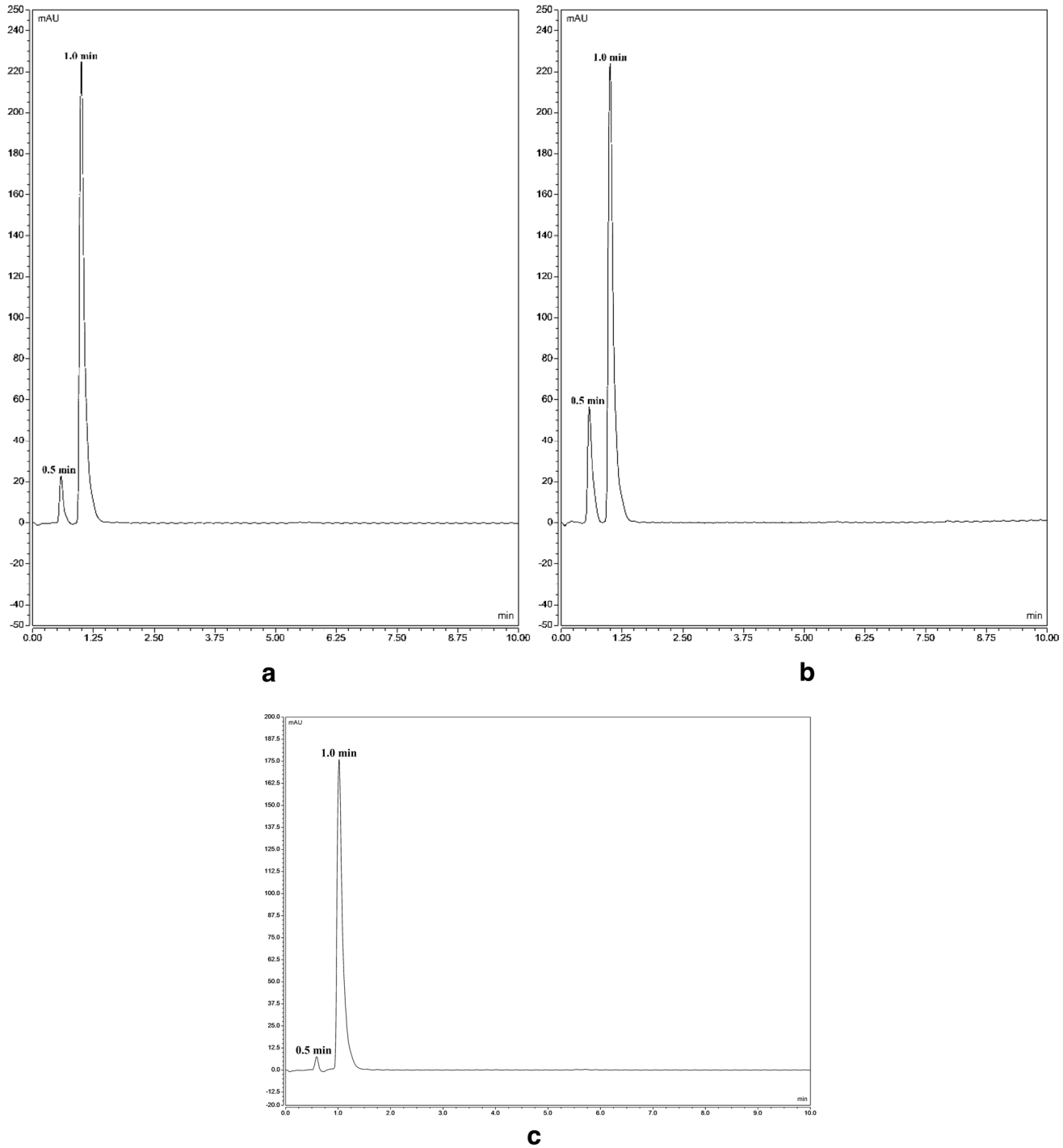


Fig. 4 **a** Chromatogram of 20 µg DAPA and its acid degradation product at 70 °C after 30 min (under the mentioned conditions "Method Development"). **b** Chromatogram of 20 µg DAPA and its base degradation product at 70 °C after 30 min (under the mentioned conditions "Method Development"). **c** Chromatogram of 20 µg DAPA and its oxidation degradation product at room temperature (under the mentioned conditions "Method Development")

Specificity

Specificity is the capability of the analytical method to determine the analyte response when accompanied with interferences including degradation products and substances used during formulation. The proposed method was used for the determination of DAPA and MET and for separation

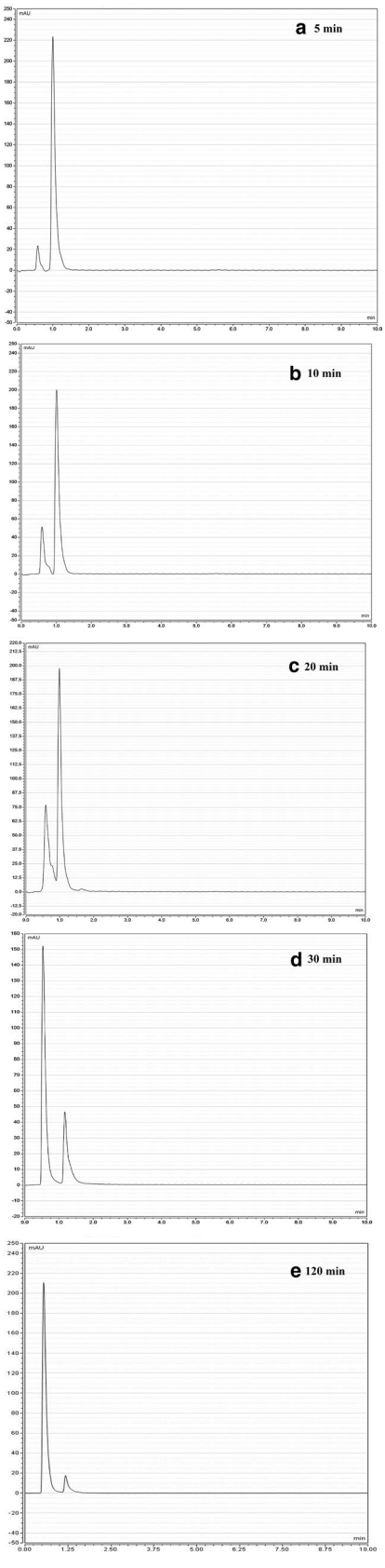


Fig. 5 Degradation pattern of 20 µg DAPA by 0.5 M NaOH at 70 °C, over time intervals of 5, 10, 20, 60,120 min. The main degradation product retention time was 0.5 and the remaining dapagliflozin appeared at retention time of 1.0 min under the mentioned conditions "Method Development"

between DAPA and its main degradation product. The chromatograms of the samples were monitored for the detection of any extra peaks, however, no chromatographic interference from any of the excipients was observed at the retention times of the drugs. Moreover, the chromatogram of each analyte in the sample solution was completely matching with the chromatogram received from the standard solution. These results revealed the absence of interference from any ingredient in the dosage form and therefore approve the specificity of the proposed method, specificity results showed in Table 2.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD was taken as the amount for which the S/N ratio was 3:1, while the LOQ was taken as the amount for which the S/N ratio was 10:1. The results are shown in Table 2.

Robustness of the Method

Robustness was verified by changing slightly some parameters of the chromatographic conditions with no effect on the resolution factor within two adjacent peaks (DAPA, MET and DAPA with its degradation product). The flow rate was changed by ± 0.1 mL/min. The wavelength was altered ± 2 nm, while the pH was changed by ± 0.2 unites. It was found that the suggested method is robust against these changes.

Application of the Method for Analysis of Laboratory Prepared Mixture and Dosage Form

The proposed method was effectively applied for the analysis of laboratory prepared binary mixtures of DAPA and MET in which the drugs were mixed in different ratios. The average percent recoveries were established on the average of triplicate determinations as in Table 4.

Additionally, Xigduo XR® tablets were analyzed to show the capability of the proposed UHPLC–UV method for routine analysis of the studied drugs in their formulation. As the ratio of two drugs in dosage form is out of the calibration curve of DAPA, there was no way to determine it without sample enrichment with known amount of its pure form. Then by subtraction of the added pure concentration from the spiked concentration, the initial amount

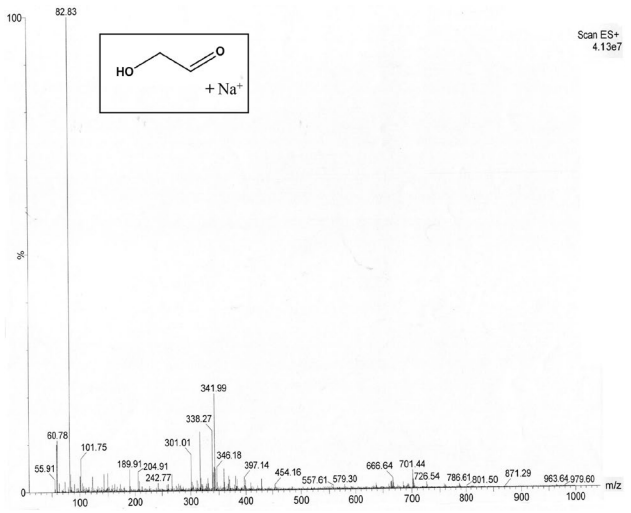
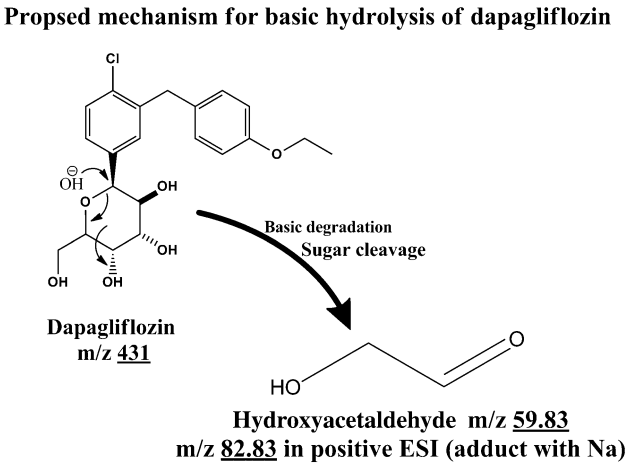


Fig. 6 Full scan spectra of $[M+H]^+$ of the basic degradation products of dapagliflozin

in tablet was found out. Moreover, this enrichment enables the study of the interference of commonly used tablet excipients as it is a sort of standard addition technique. Meanwhile, there was no need for addition of pure MET to sample as its tablet concentration was within its calibration range. Yet, MET recovery was determined for each dosage form sample along with DAPA, and it was excellent. The concentrations of the two drugs were calculated referring to the corresponding regression equation. The mean percentage recoveries as well as the standard deviation obtained from the standard addition method indicated a good precision and accuracy for the proposed UHPLC–UV method, as presented in Table 4.



Scheme 1 Proposed basic degradation of dapagliflozin

Degradation Behavior

This work presents the first kinetic stability study for DAPA using different stress conditions then measuring the rate of formation of degradates over time [37]. Values of $t_{1/2}$ and t_{90} were calculated for the molecule after accurate applying of each stress condition. The results showed initially the remarkable stability of DAPA before it degraded over time intervals from 5 min to 2 h. This stability may be referred to its glycoside scaffold, only the terminal sugar was the part affected and detached over degradation.

It was found that DAPA was susceptible to acidic, alkaline and oxidative degradation but resistant to thermal and ultraviolet effects (Fig. 4). However, it was highly susceptible to alkaline hydrolysis; the corresponding chromatographic degradation pattern at increasing time intervals is depicted in Fig. 5.

The corresponding full MS scan spectrum in Fig. 6 shows a signal with highest relative abundance (compared to other signals) of the molecular mass of the expected main degradation product. The expected degradation pathway of DAPA and structure elucidation of the main degradates in alkaline conditions are represented in Scheme 1 in which we notice cleavage of the sugar part of the glycoside giving away hydroxyacetaldehyde moiety. This was emphasized by the mass spectrum obtained from the solution suspected to alkaline degradation.

The deduced structure of the alkaline degradation product is confirmed to be of sugar origin owing to its early elution which might be referred to its polar structure. Table 5 represents examples of other reported degradation studies of DAPA, which in turn reveals that the proposed method is more advantageous due to lower concentration of acid or base used in degradation, higher degradation % coincides with the stated stress degradation % to be 20–80% besides other optimized chromatographic criteria due to UHPLC technique.

Degradation Kinetics

The kinetics of alkaline degradation of DAPA was investigated through withdrawing different samples of the degradation solutions at different time intervals (5, 10, 15, 30, 60, 120 min). A regular decrease in the drugs concentrations was detected with increasing time. When plotting the log of remained drug concentration versus time, a linear relationship was observed with good correlation coefficients (Fig. 7).

Pseudo-first-order is the term used when two reactants are involved in the reaction but one of them is in such a

Table 5 Stress conditions applied by reported methods for dapagliflozin

Drug	Method		Conditions	% degraded
Dapagliflozin	Acid hydrolysis	Proposed method	Heating with 0.1, 0.5, 1 M HCl at 70 °C for 120 min	21% using 1M HCl
		Mohammad Yunoos et al. [5]	Reflux with 2 M HCl at 60 °C for 30 min	7.49
		Manasa Sanagapati et al. [8]	Heating with 2 M HCl at 60 °C for 30 min	7.4
	Base hydrolysis	Proposed method	Heating with 0.1, 0.5, 1 M NaOH at 70 °C for 120 min	88% using 0.5M NaOH
		Mohammad Yunoos et al. [5]	Reflux with 2 M NaOH at 60 °C for 30 min	6.37
		Manasa Sanagapati et al. [8]	Heating with 2 M NaOH at 60 °C for 30 min	6.4
	Thermal hydrolysis	Proposed method	Heating at 60 °C for 72 h	21%
		Mohammad Yunoos et al. [5]	By refluxing on a water bath for 6 h at 60 °C	4.99
		Manasa Sanagapati et al. [8]	Heating at 105 °C for 6 h	4.4
	Photo stability	Proposed method	Exposing powder to UV for 24 h	No degradation
		Mohammad Yunoos et al. [5]	Exposing powder to UV	1.74
		Manasa Sanagapati et al. [12]	Exposing powder to UV for about 7 days	1.4
	Oxidative degradation	Proposed method	Drug was treated with 3% and 15% (v/v) H_2O_2 at room temperature	68% after 12 h
		Mohammad Yunoos et al. [5]	Drug was treated with 20% H_2O_2 (v/v) at 60 °C for 30 min	5.66
		Manasa Sanagapati et al. [8]	Drug was treated with 20% H_2O_2 (v/v) at 60 °C for 30 min	5.1

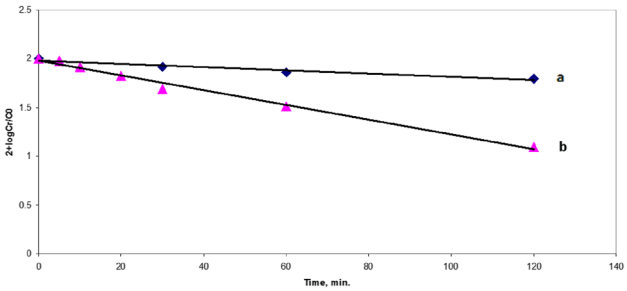


Fig. 7 Pseudo-first order kinetic plot of: **a** the acidic degradation of DAPA (20 µg/mL) at 70 °C using 1M HCl and **b** basic degradation of DAPA (20 µg/mL) at 70 °C using 0.5 M NaOH

large excess that any change in its concentration is negligible compared with the change in the concentration of the other reactant (drug). Rate constant (K), time left for 50% potency ($t_{1/2}$) and time left for 90% potency (t_{90}) under alkaline stress condition were calculated using the following equations [37]:

$$\text{Log}\left[C_t\right] = \frac{\log\left[C_0\right] - K_t}{2.303} \quad (1)$$

$$t_{1/2} = \frac{0.693}{K}, \quad (2)$$

$$t_{90} = \frac{0.105}{K}, \quad (3)$$

where K is the rate constant, $[C_0]$ is the concentration of DAPA at time $t=0$ and $[C_t]$ is its concentration at time t. The calculated K per minute, $t_{1/2}$ and t_{90} values are shown in Table 6 under various stress degradation conditions.

Stressful degradation was observed under alkaline hydrolysis conditions for DAPA at 0.5N NaOH, where the K value was found to be the highest compared with acidic and oxidative degradations. On contrary, both $t_{1/2}$ and t_{90} at alkaline conditions were found to be lower than those of other conditions.

Table 6 The calculated kinetic values of DAPA degradation under various stress conditions

Condition	Alkaline degradation			Acid degradation			Oxidative degradation	
	0.1 N	0.5 N	1.0 N	0.1 N	0.5 N	1.0 N	5 h	12 h
Value								
K per minute	No quantitative degradation	0.023	Complete degradation	No quantitative degradation		0.0053	0.000155	0.001574
$t_{1/2}$ (min)		29.39				129.64	4460.018	440.2305
t_{90} (min)		4.454				19.644	675.7603	66.70159

Conclusion

A novel, simple and specific stability-indicating UHPLC–UV method was developed for the simultaneous determination of dapagliflozin (DAPA) and metformin (MET) in pharmaceutical dosage forms followed by studying DAPA degradation under different stress conditions. Degradation reaction kinetics was investigated in addition to structural elucidation of the main degradation product. The utilization of UHPLC has enabled the use of a short column with small particle size which resulted in uniform chromatographic peaks within a very short run time. In addition, the low flow rate applied in this method has offered less solvent consumption which is considered to be cost effective and eco-friendly.

The method was validated in accordance with ICH guidelines. The results gained from the validation study have confirmed that the new method is selective, linear, precise and accurate. Moreover, for the first time, degradation kinetics of DAPA was studied, proving that degradation was a pseudo-first-order reaction, and it was concluded that DAPA was extensively degraded under basic conditions. Based on all previous advantages and results, the developed method could be conveniently used in quality control laboratories.

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Compliance with ethical standards

Conflict of interest No conflict of interest of any kind.

Research involving human or animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

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