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ORIGINAL

Kinetic Degradation Study of Ipragliflozin Coupled with MS/MS Structural Elucidation

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Abstract

Ipragliflozin degradation behavior was studied under different conditions: acidic, basic, photolytic, oxidative and thermal degradation conditions. This forced degradation study showed the extensive degradation of Ipragliflozin under acidic, basic and oxidative conditions while showed high stability under thermal and photo-degradation conditions. The separation of Ipragliflozin and its degradation products was done using Hypersil Gold® UPLC C18 column with 1.9 µm particle size (3 × 50 mm) as stationary phase and a mobile phase composed of acetonitrile: potassium monobasic phosphate buffer pH 3 (50:50; v/v) delivered at flow rate of 0.6 mL min^{-1}. Validation of the proposed method was carried out in accordance to the International Council for Harmonisation's guidelines. The method was found to be linear within the concentration range 5.0–50.0 µg mL^{-1} with a limit of detection of 1.48 µg mL^{-1}. Accuracy was proven as the percentage recovery was 98.57 ± 0.40 and the percentage relative standard deviation was 0.82 which ascertained the precision. After chromatographic separation, mass characterization was used to structurally elucidate the degradation products and to propose the degradation pathway. Kinetics parameters of oxidative, acidic and basic degradation processes were determined and it was demonstrated that the degradation appears to follow a pseudo-first-order reaction. The simplicity and sensitivity of the proposed method promote its regular use in quality control laboratories.

Keywords Ipragliflozin · UPLC · Forced degradation · MS/MS · Validation

Introduction:

Ipragliflozin (IPG), Fig. 1, is an orally administered antihyperglucaemic medication to be used as monotherapy or in conjunction with another class of antidiabetic agents. IPG prevents glucose reabsorption in the kidneys by inhibiting sodium-glucose co-transporter-2 (SGLT2), which is located in the proximal convoluted tubules so by inhibiting that transporter, the glucose excretion in urine is facilitated. By excreting glucose, blood glucose level falls improving all glycemic parameters [1–4]. It is important to emphasize that the advantage of this class is to have a mode of action which is totally separate and distinct from the actions of insulin and it depends only on plasma glucose levels leading to the reduction of the potential for hypoglycemia and no risk of overstimulation of pancreatic β-cells [4, 5].

By reviewing the literature, it was found that IPG was estimated by only a few analytical methods as high performance liquid chromatography with ultraviolet detection [6–8], ultra-performance liquid chromatography [9], LC–MS/MS [10–12], spectrophotometry [13] and voltammetry [14]. These methods were developed for the estimation of IPG in tablet and pure form.

Forced degradation studies demonstrate the active pharmaceutical ingredient's chemical stability, which is a major concern because it affects the drug's safety and efficacy. Additionally, the determination of the drug's stability aids in the selection of appropriate formulation and packaging, as well as the requirements of suitable storage conditions and

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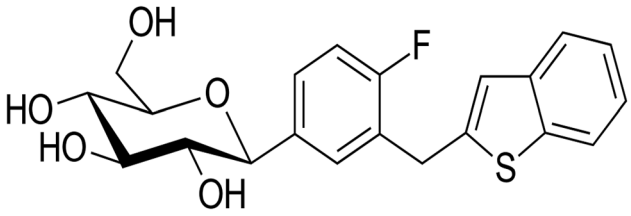


Fig. 1 Chemical structure of Ipragliflozin

shelf life. Forced degradation refers to the degradation of a drug material or drug product under conditions that are more intense than those encountered in accelerated degradation, generating the degradation products that can be evaluated to assess the drug's stability [15].

It is important to mention also that the presence of the degradation products as kind of impurities could be hazardous to public health; and therefore they must be identified and well-studied to ensure the quality and safety of drugs. The reversed phase high-performance liquid chromatography (HPLC) is one of the most widely used analytical techniques for separating, quantifying impurities and many other applications [16]. The ultra-performance liquid chromatography (UPLC) has many advantages over HPLC as shortening the time of analysis and decreasing the solvent consumption leading to analysis cost reduction [16]. Furthermore, LC–MS has been employed in a variety of pharmaceutical studies especially the characterization of the degradation products of various drugs [17–23].

As far as we know, there is no reported method in the literature for the identification and structural elucidation of degradation products of IPG. Therefore, the main target of this study is to implement and validate a novel UPLC method for separation of IPG and its degradation products followed by the characterization of its degradation products using MS/MS. This UPLC method can be consequently used for the determination of the drug in many different matrices. Additionally, a kinetic study for the degradation of IPG has been conducted and its kinetic parameters as K, t_{90} and $t_{1/2}$ have been reported for the first time.

Experimental

Chemicals and Reagents

Ipragliflozin pure substance guaranteed and certified to contain 99.90% ± 0.01 was obtained from BaoJi Guokang Biotechnology co., Ltd, and the purity was confirmed by mass spectrometric analysis. HPLC grade orthophosphoric acid (85%) and acetonitrile were bought from Fisher Scientific (Loughborough, Leicestershire, UK). Monobasic potassium phosphate and sodium hydroxide of analytical grade were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). Hydrochloric acid and hydrogen peroxide of analytical grade were purchased from SD Fine Chem Limited (Mumbai, India). MilliQ water and distilled water were provided in house.

Instruments

A thermo Fisher UHPLC Dionex Ultimate 3000 (Germering, Germany) supplied with chromeleon 6.8 (Germering, Germany) was used. The pump (ISO-3100SD), connected to an autosampler (WPS 3000 SL) were utilized. The column temperature was maintained using column thermostat (TCC-3000SD) and the detection was relying on photo diode array detector (PDA-3000 RS) (Germering, Germany). Chromatographic separation was carried out using Hypersil Gold® UPLC C18 column with 1.9 µm particle size (3 × 50 mm) as a stationary phase. WATERS Acquity Triple quadrupole mass spectrometer (USA) was employed during the structural elucidation step. Solvent degassing was carried out utilizing Elmasonic S 60 H water bath sonicator (Germany). pH-meter (Jenway pH-meter 3310, Dunmow, Essex, United Kingdom) was employed to adjust the pH. During the degradation studies, the temperature was kept at 70 °C utilizing thermostatic water bath (Wisd—WSB-18, Berlin, Germany). Water purification system (Thermo scientific Barnstead Smart 2 Pure 3 UV, Hungary) was used to provide ultrapure water. Oven (Binder, Germany) was used to test the thermal stability of the drug. To subject the drug to photolytic stress conditions, UV lamp of 8 W power (UVP, Upland, USA) was used. A rotary vacuum concentrator linked to a vacuum pump (DVP TYRO 12, Germany), a solvent trap (CHRIST CT 02–50, Germany) in addition to a rotor (CHRIST RVC 2–18 CDplus, Germany) was used for the evaporation of the degradation samples.

Chromatographic and MS/MS Conditions

Separation was carried out using Hypersil Gold UPLC C18 column (1.9 µm particle size, 3 × 50 mm) as a stationary phase. The mobile phase composition was: 25 mM monobasic potassium phosphate, pH 3, adjusted using orthophosphoric acid and acetonitrile in the ratio of 50:50 (v/v). The detection was performed at wavelength of 230 nm where the maximum sensitivity was recorded. Preparation of the mobile phase was followed by filtration utilizing membrane filters of 0.2-µm pore size then, degassing for 20 min. The mobile phase was delivered at 0.6 mL min^{-1} flow rate. 10 µL of the sample was injected and the column temperature was kept at 30 °C.

To perform MS/MS, samples of the acidic and basic degradation collected after 2 h and sample of the oxidative degradation collected after 12 h were evaporated to dryness

using the rotary vacuum concentrator followed by reconstitution in methanol. MS/MS analysis was carried out in offline mode using Acquity triple quadrupole mass spectrometer operating in the positive ion mode. Capillary voltage was 3 kV and the sampling cone voltage was 20 V. The source temperature was 120 °C while the desolvation temperature was 400 °C with the desolvation gas flow rate of 600 L h^{-1}.

Procedures

Preparation of Stock Standard Solutions

Stock standard solution of 1.0 mg mL^{-1} of IPG was prepared by accurately weighing and dissolving 100.0 mg of IPG in 100.0 mL methanol. A working standard solution of 100.0 µg mL^{-1} was obtained by appropriate dilution of the stock standard solution using the mobile phase.

Calibration Curve Construction

Several aliquots of IPG equivalent to 50.0–500.0 µg were accurately transferred from working standard solution and transferred into a series of 10 mL volumetric flasks, the volumes were completed to the mark using the mobile phase to reach final concentrations of 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 µg mL^{-1}. A volume of 10 µL of each concentration was injected in triplicates by applying the previously mentioned chromatographic conditions. By plotting peak areas versus the corresponding concentrations, calibration curve and regression equation were obtained.

Validation of Analytical Method

In order to ensure that the proposed method is appropriate for its predetermined use, it has been validated based on the ICH guidelines regarding accuracy, linearity, precision, specificity, robustness, limits of detection and quantification. [24].

Linearity

Under the optimum chromatographic conditions, linearity was assessed by evaluating six different concentrations of IPG in triplicates. The obtained peak areas were found to be linear with the corresponding concentrations.

Accuracy

The accuracy of the analytical method is indicated by the closeness of agreement between the true and the predicted values. It is reported as percent recovery of various concentrations of standard solutions of IPG.

$$\frac{\text{Found concentration}}{\text{Prepared concentration}} \times 100$$

Precision

Intraday and interday precision were evaluated. The intraday precision is usually evaluated through the determination of three levels of the analyte on the same day. However, the interday precision is evaluated by estimating three different levels of the drug on three consecutive days. Results were calculated and interpretation was by % relative standard deviation (% RSD).

Specificity

Specificity is expressed as the capability of the analytical procedure to determine the drug of interest in presence of any interference as its degradation products. The obtained chromatograms were examined for any additional peaks leading to chromatographic interference at the retention time of the drug.

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

LOD is the lowest concentration that the method can detect but not necessarily measured as an exact value whereas, LOQ is the lowest concentration that could be quantified precisely and accurately. These parameters were determined by following the equations:

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

Robustness

Robustness is generally evaluated by proving that the method is unaffected after deliberately changing some chromatographic factors. We have changed some conditions as temperature (30 °C ± 1), percentage of aqueous to organic phase of the mobile phase (buffer: ACN, 50:50 ± 1), pH of the buffer used (3 ± 0.1), wavelength of detection (230 nm ± 2) and flow rate (0.6 mL min^{-1} ± 0.1).

Forced Degradation Studies

Forced degradation studies of bulk drug were carried out according to the ICH regulatory guidelines [25]. Acid, basic,

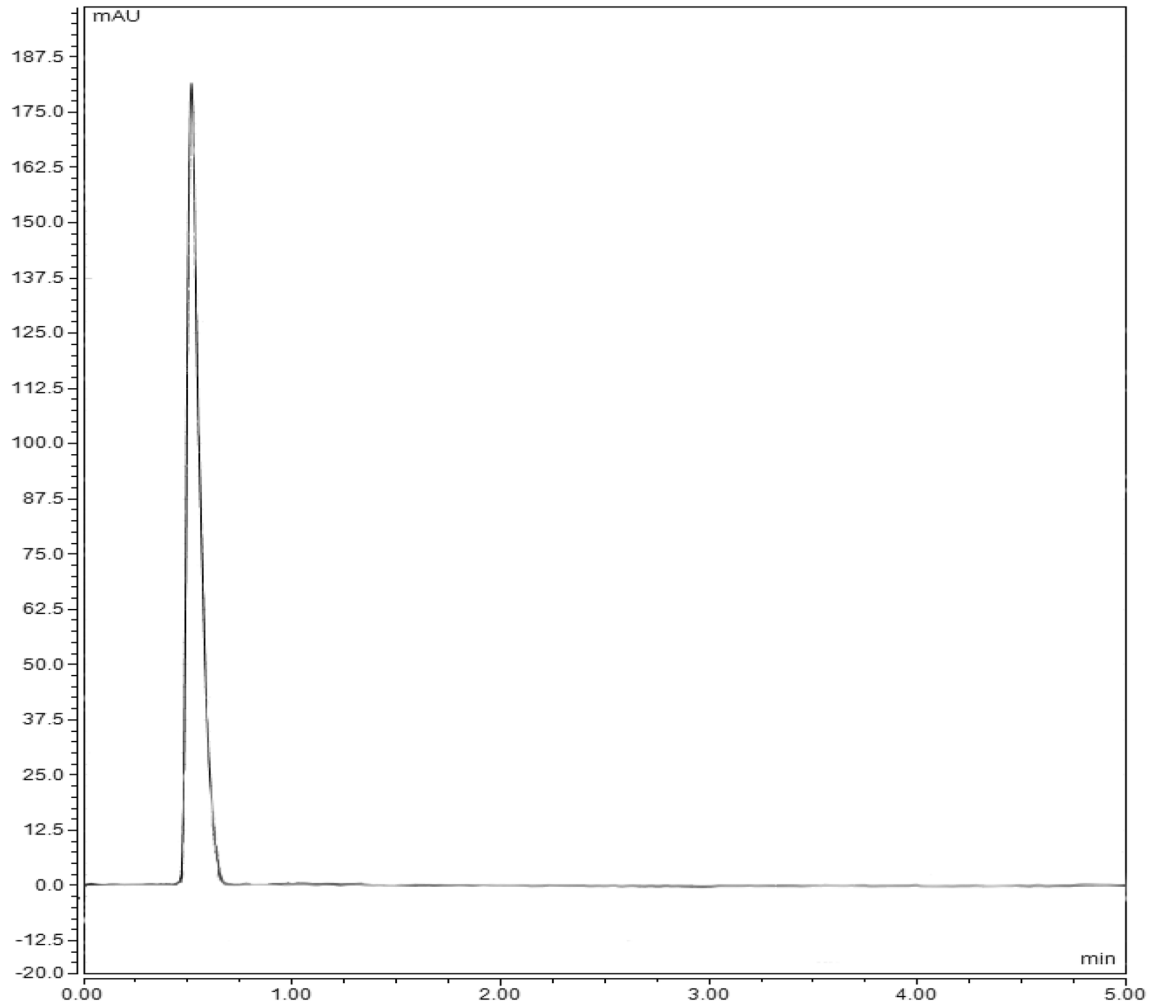


Fig. 2 UPLC Chromatogram of 20 µg mL^{-1} of Ipragliflozin under the mentioned chromatographic conditions

Table 1 Validation parameters for the estimation of Ipragliflozin by the proposed method

Parameters	Ipragliflozin
Retention time	0.6 min
Wavelength	230 nm
Linearity range	5.0–50.0 µg mL^{-1}
Slope	1.3
Intercept	0.4466
Correlation coefficient	0.9995
Accuracy (mean ± SD)	98.57 ± 0.40
LOD (µg mL^{-1})	1.48
LOQ (µg mL^{-1})	4.49

oxidative, thermal, and photolytic degradation conditions were all applied to the studied drug.

Table 2 Results of the repeatability performed on the same day by the proposed method

Concentration	Mean peak area	Standard deviation	% RSD
5.0 µg mL^{-1}	6.83	0.018	0.26
25.0 µg mL^{-1}	30.66	0.193	0.63
50.0 µg mL^{-1}	65.74	0.160	0.24

Acid and Base-Induced Hydrolysis

Suitable volumes of 10 M hydrochloric acid were added to 5 mL of methanolic working standard solution in 25 mL volumetric flasks to obtain 0.1 M, 0.5 M and 1 M solutions then those solutions were kept for 2 h in the thermostatic water bath at 70 °C. Degradation was measured at specified time intervals 5, 10, 20, 30, 60 and 120 min after being neutralized with sodium hydroxide. The volume was completed

Table 3 Results of the intermediate precision performed on three different days by the proposed method

Concentration	Replicate peak area			Mean peak area	Standard deviation	% RSD
5.0 µg mL^{-1}				6.84	0.06	0.93
Day 1	6.82	6.85	6.83			
Day 2	6.86	6.82	6.92			
Day 3	6.93	6.71	6.83			
25.0 µg mL^{-1}				30.65	0.17	0.58
Day 1	30.82	30.45	30.73			
Day 2	30.48	30.74	30.52			
Day 3	30.72	30.48	30.95			
50.0 µg mL^{-1}				65.56	0.61	0.93
Day 1	65.59	65.91	65.75			
Day 2	65.60	64.58	65.76			
Day 3	66.43	65.91	64.59			

Table 4 Results of the robustness by deliberately changing column temperature, mobile phase composition, wavelength of detection, pH of the buffer and flow rate of the proposed method

Parameter	Value	Mean	SD	% RSD
Temperature 30 °C ± 1	31 °C	21.11	0.31	1.45
	29 °C			
Mobile phase composition % aqueous: organic ±1%	51:49	21.29	0.41	1.92
	49:51			
Wavelength used 230 nm ± 2	232 nm	20.89	0.41	1.96
	228 nm			
pH 3 ± 0.1	3.1	22.42	0.20	0.91
	2.9			
Flow rate 0.6 mL min^{-1} ± 0.1	0.7 mL min^{-1}	21.13	0.36	1.69
	0.5 mL min^{-1}			

with mobile phase. For base induced hydrolysis, same procedure was performed using 10 M sodium hydroxide to obtain same molarities and heated in the water bath. Degradation was measured in the time intervals after being neutralized using hydrochloric acid and the volume was completed using acetonitrile to inhibit the salting out of the phosphate buffer included in the mobile phase.

Oxidative Degradation

Suitable volume of 30% hydrogen peroxide was added to 5 mL of methanolic working standard solution in 25 mL volumetric flasks to obtain 3% and 15% concentrations of hydrogen peroxide after dilution. Degradation was measured at specified time intervals 15, 30, 45, 60 and 120 min after dilution with water to stop the action of hydrogen peroxide.

Thermal Degradation

An accurately weighed 5.0 mg of IPG solid powder was kept in oven for 72 h at 55 °C then cooled and dissolved in mobile phase to yield final concentration of 20 µg mL^{-1}.

Photolytic Degradation

Photolytic stress study was performed by accurately weighing 5.0 mg of drug solid powder, exposing it for 72 h to ultraviolet lamp of 8 W power then dissolving it in mobile phase to obtain same concentration.

Results and Discussion

The main aspect in the proposed study is to study the kinetics parameters of IPG degradation under susceptible stress conditions considering the degradation % should be between 20 and 80% of the studied drug.

Optimization of Chromatographic Conditions

To achieve the main purpose of this study, there was a necessity to implement and validate a method for separation of IPG and its degradation products. Different factors have been studied including the type of the column, the composition of the mobile phase, flow rate and wavelength of detection. Regarding the column, two columns with different particle size; Hypersil Gold UPLC C18 column (1.9 µm particle size, 3 × 50 mm) and BDS Hypersil C18 (3 µm particle size, 3 × 100 mm) have been tried. Both columns showed comparable results regarding the separation and the resolution but favorable experimental

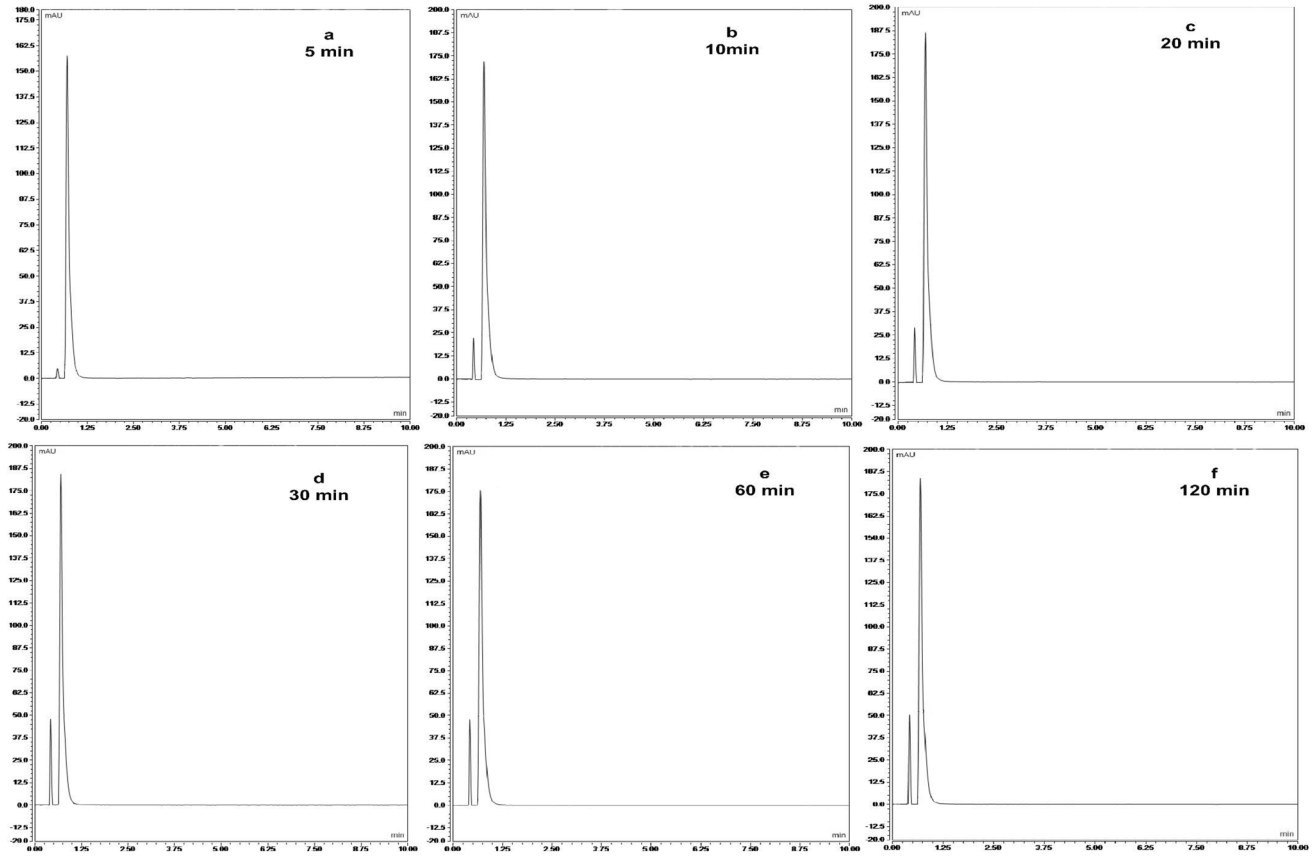


Fig. 3 LC chromatograms showing the degradation pattern of 20 µg mL^{-1} ipragliflozin after exposure to 1 M HCl, at 5, 10, 20, 30, 60, 120 min time intervals at 70 °C. Under the mentioned optimum conditions, the main degradation product retention eluted at 0.4 min and the remaining ipragliflozin eluted at retention time of 0.6 min

parameters as flow rate and mobile phase consumption moreover the sensitivity of the first column was significantly higher than the second one. Regarding the mobile phase, two systems, water: acetonitrile and water: methanol, have been tried in different ratios but the separations were not satisfactory. Then buffer was added with different ratios where the optimum mobile phase composition showing the best results was acetonitrile: 25 mM phosphate buffer (50:50, v/v). Moreover, the effect of pH was investigated where pH 3 gave the best results. By changing the flow rate, the one that leads to the best separation was 0.6 mL min^{-1}. The last factor was the wavelength, 230 nm was chosen due to its good sensitivity for both the drug and its degradation products. Chromatograms of the different trials are shown Fig. S1, S2 and S3 and a typical chromatogram showing IPG being eluted at 0.6 min is shown in Fig. 2.

Method Validation

Linearity

The method was linear in the range of 5.0–50.0 µg mL^{-1}, with a correlation coefficient of 0.9995, under the optimum chromatographic conditions. Results are represented in Table 1.

Accuracy

The suggested method was demonstrated to be accurate evidenced by %recovery of three levels of standard solutions of IPG in triplicates. Results are represented in Table 1.

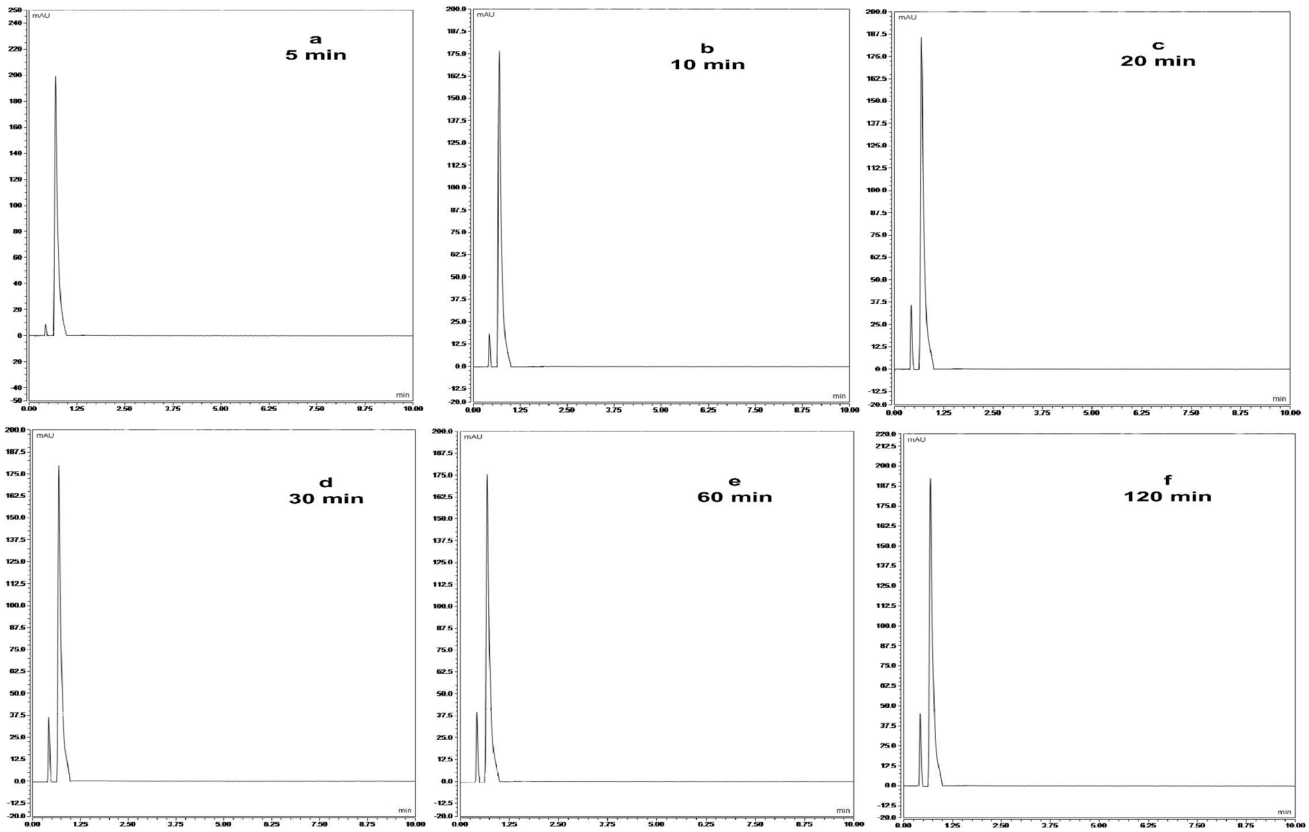


Fig. 4 LC chromatograms showing the degradation pattern of 20 µg mL^{-1} Ipragliflozin after exposure to 1 M NaOH, at 5, 10, 20, 30, 60, 120 min time intervals at 70 °C. Under the mentioned optimum conditions, the main degradation product eluted at 0.4 min and the remaining ipragliflozin eluted at retention time of 0.6 min

Precision

The calculated %RSD values were less than 2% after evaluating the intraday and interday precision, showing that the procedure was precise. The % RSD were calculated and shown in Tables 2 and 3.

Specificity

The proposed method has demonstrated to be specific by being efficiently used for the determination and separation between IPG and its degradation product with sharp uniform peaks.

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

LOD and LOQ were computed using the previously mentioned equations and the results are in Table 1.

Robustness

The calculated % RSD values were found less than 2%. Results are reported in Table 4.

Degradation Behavior

After exposing the drug to the previously mentioned stress conditions under experimental section, it was observed that IPG is susceptible to acidic, basic and oxidative degradation and resistant to thermal and photolytic degradation.

The chromatograms of both basic and acidic degradation showed the appearance of additional peak of the degradation product of which the peak area increases by time and by increasing the molarities of the acid or base accompanied by the decrease in the peak area of the drug. Typical chromatograms of acidic and basic degradation study are shown in Figs. 3 and 4, respectively.

The oxidative degradation showed the most extensive degradation of the drug may be due to the presence of sulfur-containing structure. This high susceptibility to the oxidative degradation was an obstacle to study the kinetics of such

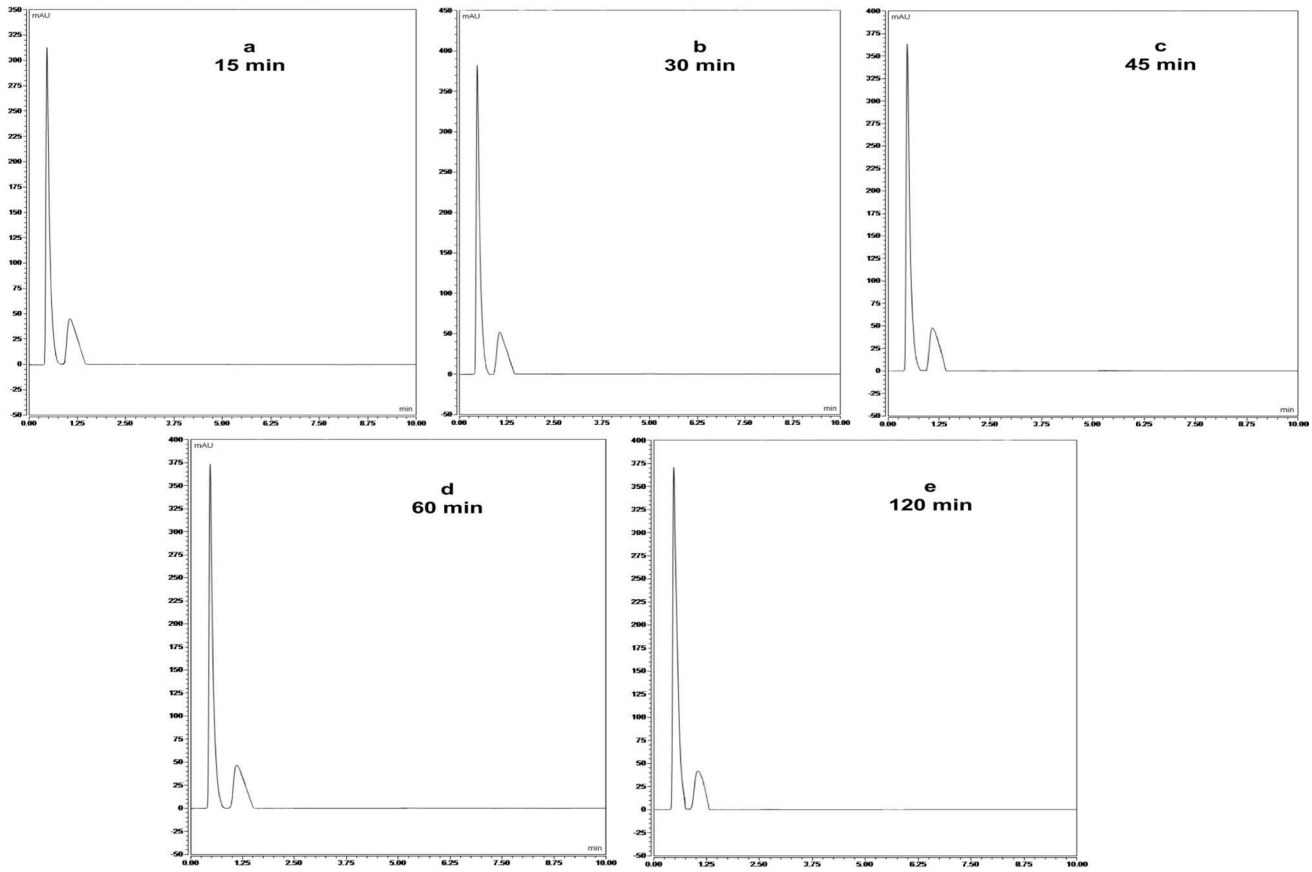


Fig. 5 LC chromatograms showing the degradation pattern of 20 µg mL^{-1} Ipragliflozin after exposure to 3% H_2O_2 at room temperature, at 15, 30, 45, 60, 120 min time intervals. Under the mentioned optimum conditions, the main degradation product eluted at 0.4 min and the remaining ipragliflozin eluted at retention time of 0.9 min

degradation behavior along different time intervals. Typical chromatograms of oxidative degradation study are shown in Fig. 5.

The chromatograms of thermal and photolytic degradation showed no additional peaks to the drug's peak, Fig. 6. One previously reported stability indicating method of IPG [8], was compared with the proposed method regarding stress conditions and % degradation as depicted in Table 5.

Kinetics parameters were evaluated for acidic, basic and oxidative degradation processes. Long exposure time under these specified conditions were avoided to give chance for gradual decrease in the peak area of the intact drug. Therefore, rate constant (K), $t_{1/2}$ and t_{90} were estimated after 30 min exposure time for acid, base and oxidative stress conditions as shown in Table 6 and Fig. 7. Through substitution in the following Eqs. (1–3), K, $t_{1/2}$ and t_{90} were calculated. Within the studied period of time (30 min), the calculated factors for acidic degradation pathway showed relatively higher values than the basic ones. Meanwhile, the basic degradation pathway results were higher than the oxidative ones.

$$\text{Log}\left[C_t\right] = \log\left[C_0\right] - Kt/2.303, \tag{1}$$

$$t_{1/2} = 0.693 / K, \tag{2}$$

$$t_{90} = 0.105/K. \tag{3}$$

Moreover, the % degradation was estimated for the three pathways after 2 h of exposure to acid, base hydrolysis and after 12 h for oxidative degradation, as shown in Table 5.

MS/MS Characterization of IPG and Its Degradation Products

Upon mass spectrometric scanning of the different degradation samples (after exposing to acidic, basic and oxidative stress conditions), identification of degradation products m/z values were achieved, Fig. 8a–c. Fragmentation of the obtained masses was performed to get close view about the structure of the degradation products using MS/MS mode. Consequently, structures of the degradation products were

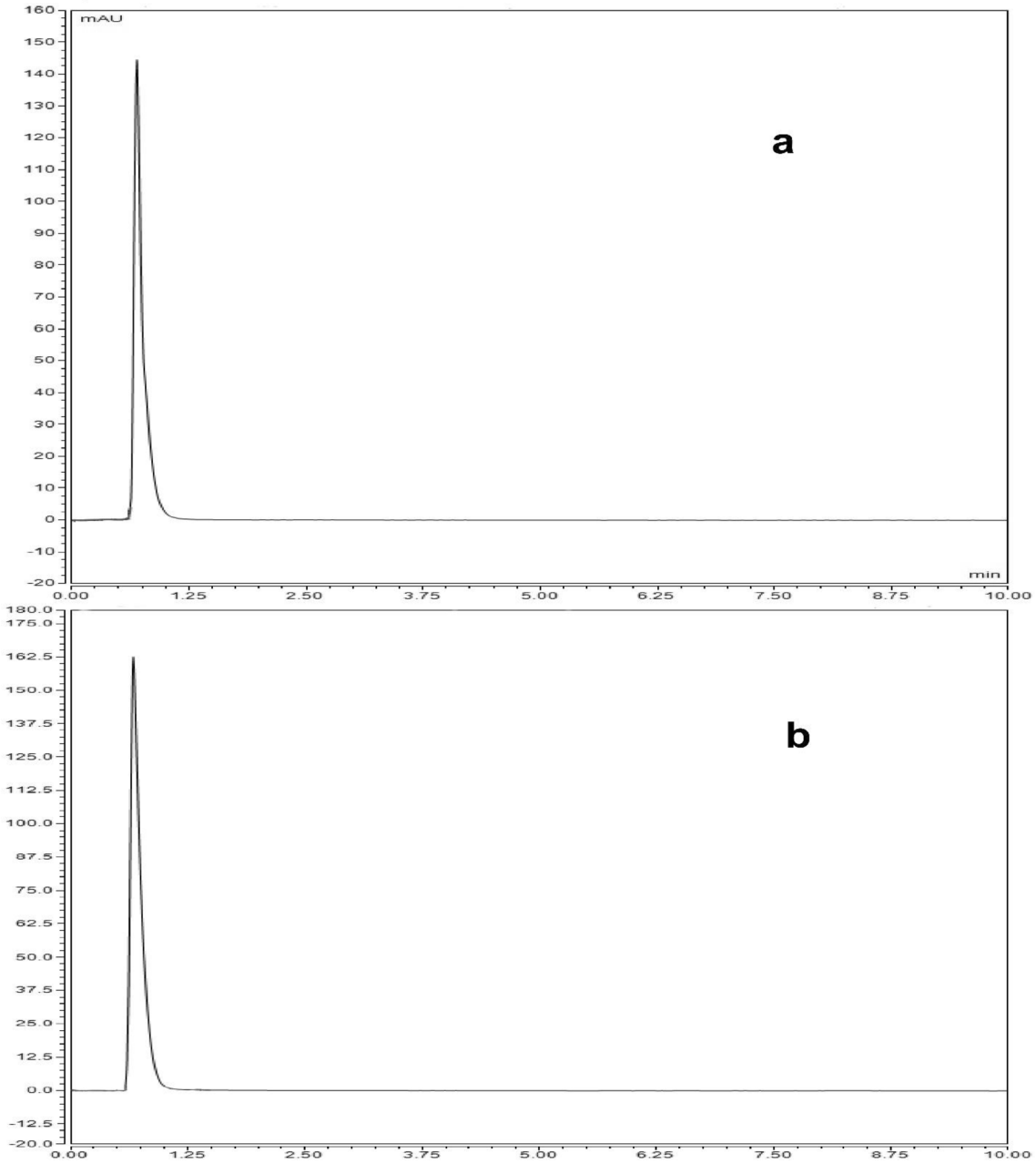


Fig. 6 Chromatograms representing 20 µg mL^{-1} of Ipragliflozin after exposure to **a** thermal and **b** photolytic stress conditions under the mentioned chromatographic conditions

characterized depending on the obtained mass data, Fig. S4a, b, c.

The structural elucidation was performed in ESI–MS positive ion mode. The mass spectrum showed the highest relative abundance (especially in contrast to other signals) of the expected major degradation product $[M+H]^+$ ion at *m/z* 217. Scheme 1 shows the suggested degradation pathway of IPG and the structural elucidation of the major degradation product, which involves the detachment of benzothiophene producing 2-[4-fluoro-3-(hydroxymethyl) phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol. In the mass spectrometer, the cleavage of the sugar part of the glycoside by McLafferty rearrangement took place, giving away 1-propene-1,3-diol moiety and 1-[4-fluoro-3-(hydroxymethyl) phenyl]propane-1,2,3-triol, as shown in Scheme 2. The degradation product's elucidated structure suggests to be of sugar origin, mainly attributed to its early elution, that could be interpreted by its polar structure [26].

Table 5 Comparison of stress conditions employed by the suggested and reported method [8] for Ipragliflozin

Type of degradation	Method	Conditions applied	% Degraded
Acid hydrolysis	Suggested method	0.1 M, 0.5 M and 1 M of HCl at 70 °C for 2 h	27.49 using 1 M HCl
	Reported method [8]	0.1 N HCl at room temperature for 24 h	12.21
Base hydrolysis	Suggested method	0.1 M, 0.5 M and 1 M of NaOH at 70 °C for 2 h	33.37 using 1 M NaOH
	Reported method [8]	0.1 N NaOH at room temperature for 24 h	9.43
Oxidative degradation	Suggested method	3% and 15% of H_2O_2 at room temperature for 12 h	54.42 using 15% H_2O_2
	Reported method [8]	10% H_2O_2 at room temperature for 12 h	24.5
Thermal degradation	Suggested method	Thermal at 55 °C for 72 h	0
	Reported method [8]	Thermal at 80 °C for 24 h	0
Photodegradation	Suggested method	Exposure to ultraviolet lamp of 8 W power for 72 h	0
	Reported method [8]	Sunlight for 24 h	0

Table 6 The computed kinetic parameters of Ipragliflozin degradation under acidic, basic and oxidative stress conditions

Kinetics parameters	Acid hydrolysis (0.1 M)	Base hydrolysis (0.1 M)	Oxidative (3% H_2O_2)
K per min	0.016897	0.010302	0.008324
$t_{1/2}$ (min)	40.83	67.26	83.24
t_{90} (min)	6.21	10.19	12.61

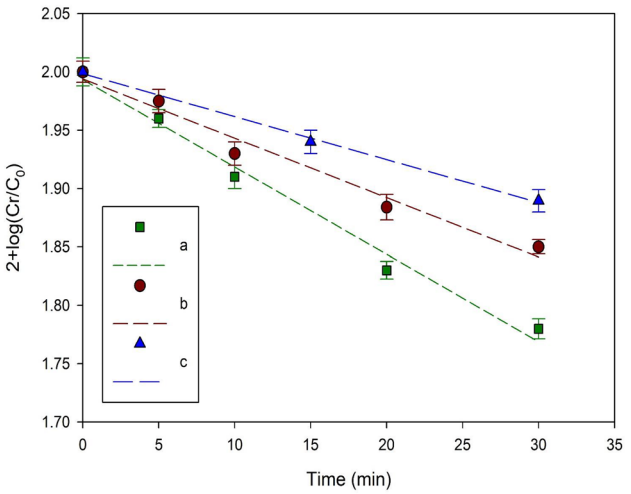


Fig. 7 Pseudo-first order kinetic plots of the degradation of 20 µg mL^{-1} Ipragliflozin: **a** acidic degradation using 0.1 M HCl at 70 °C, **b** basic degradation using 0.1 M NaOH at 70 °C and c-oxidative degradation using 3% H_2O_2

Conclusion

A new, accurate and precise stability-indicating UPLC method coupled with kinetic study was implemented to study the degradation behavior of Ipragliflozin. The drug was exposed to acid, alkali, oxidative, thermal and photolytic degradation. It was found that the drug is affected by the acidic and basic degradation due to the presence of terminal sugar part which is affected and detached over degradation. Also it is highly susceptible to oxidative degradation due to the presence of sulfur containing structure. The method was successfully validated based on the ICH guidelines. After the separation of the degradation products, structural elucidation using MS/MS was performed for the first time and it was possible to suggest a pathway of the drug's degradation. The proposed UPLC stability-indicating method has many advantages over the reported HPLC method as the shortening of analysis time and the decrease in solvent consumption and can be furtherly used for the detection of the drug in other matrices. This research work provides useful data for future studies on Ipragliflozin as there are limited data reported in the literature.

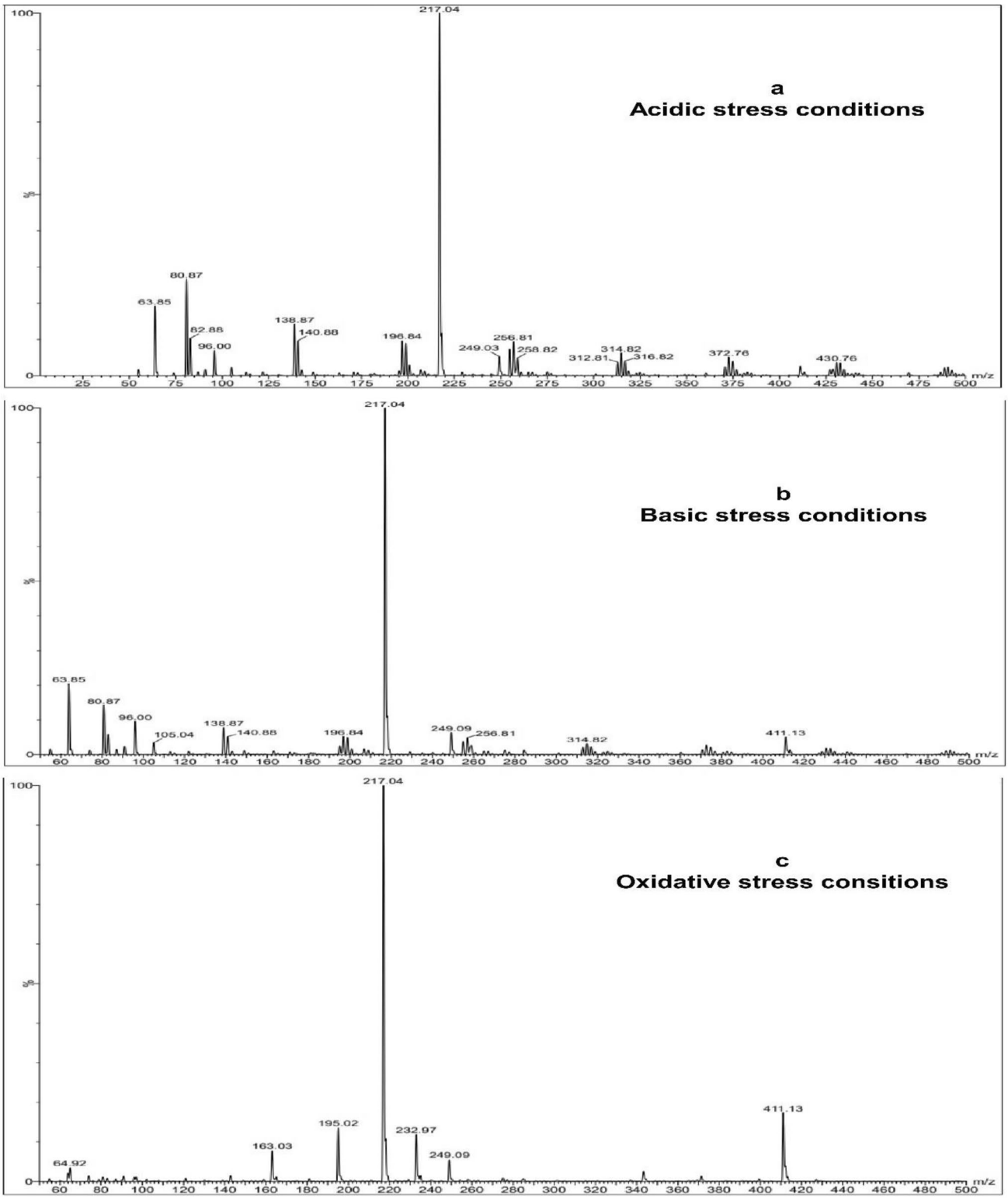
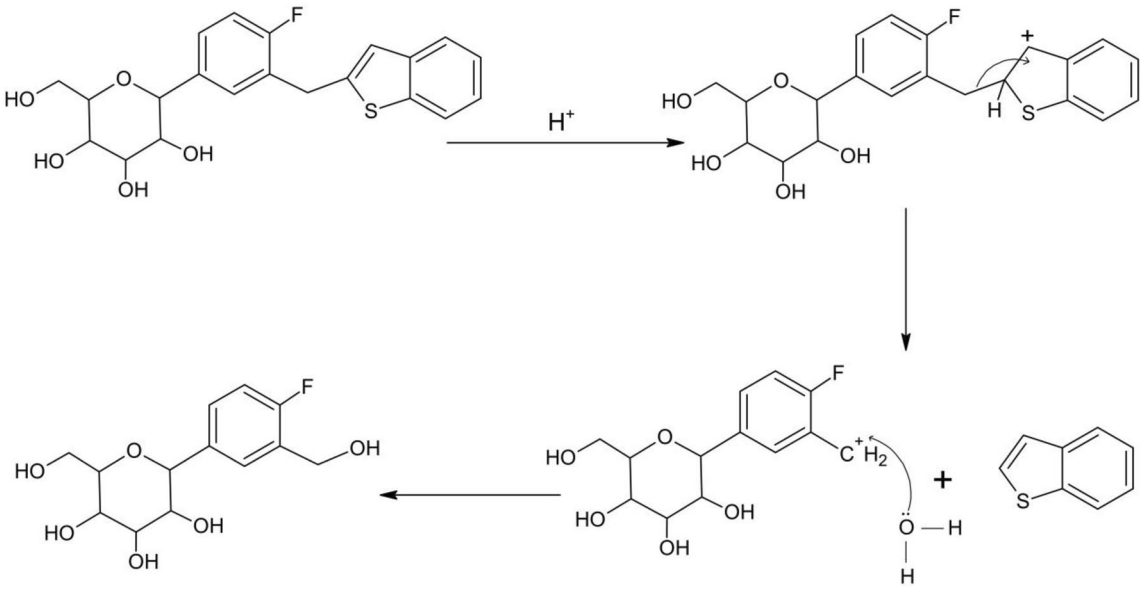
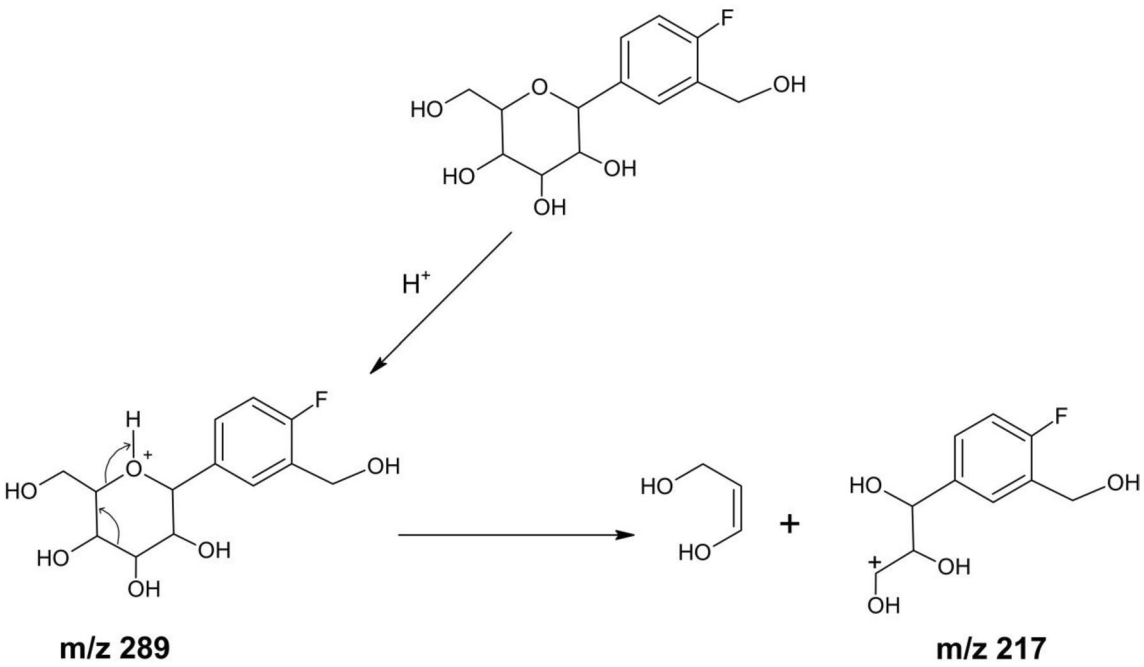


Fig. 8 Full mass scan spectra of $[M+H]^+$ of the **a** acidic **b** basic and **c** oxidative degradation products of ipragliflozin



Scheme 1 Proposed acidic degradation pathway of ipragliflozin



Scheme 2 Suggested fragmentation pathway of ipragliflozin and its degradation product $[M+H]^+$

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Declarations

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