

Research Article

STABILITY-INDICATING HPLC-DAD METHOD FOR THE DETERMINATION OF IPRAGLIFLOZINE

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ABSTRACT

Introduction: A novel stability-indicative of RP-HPLC method was developed and validated for the quantitative estimation of ipragliflozin in bulk drugs and pharmaceutical dosage form in the presence of its forced degradation products. The drug was subjected to variable stress conditions including hydrolysis, oxidation, thermal and photolysis. The drug was found to be labile to acidic hydrolysis, basic hydrolysis, oxidation but stable in thermal, photolysis and neutral hydrolytic conditions. **Experimental:** Successful chromatographic separation of ipragliflozin was achieved on HAMILTON column prp-x100 C18 column at a flow rate of 1 mL/min using mobile phase composition of methanol : acetonitrile : Water in ratio of (50: 25 : 25, by volume). The eluents were monitored by the diode array detector and peak area values were measured at 230 nm. The validity of the method was assessed by evaluating accuracy, precision, specificity and robustness. **Results and discussion:** The linear regression analysis data for the calibration curve shows a good relationship in the range of 10 – 100 µg/mL. **Conclusions:** The developed method was successfully applied for the estimation of ipragliflozin in its commercial dosage form and could be used for the routine analysis of the studied drug in quality control laboratories.

Keywords: Ipragliflozine; HPLC-DAD; Stability-indicating assay; Degradation product.

INTRODUCTION

Ipragliflozine, {(1S)-1,5-anhydro-1-C - { 3 - [(1 - benzothiophen - 2 - yl) methyl] -4-fluorophenyl}-D-glucitol}, (Suglat®, Fig. 1) is a highly potent and selective sodium-dependent glucose co-transporter-2 (SGLT2) inhibitor, a novel class of hypoglycemic agents [1-4]. It is white powder practically insoluble in water and propylene glycol; very slightly soluble in ethanol; slightly soluble in acetone and soluble in methanol [5]. SGLT2 inhibitors suppress glucose re-absorption in the proximal tubules of the kidney and promote the urinary excretion of glucose; therefore, SGLT2 inhibitors may reduce blood glucose levels and bodyweight [4]. Tahara et al. previously reported that ipragliflozin improved diabetes/obesity-associated metabolic abnormalities in type 2 diabetic mice, which suggests that ipragliflozin may be valuable in the treatment of type 2 diabetes with metabolic syndrome [5]. Additional advantages of SGLT2 inhibitors are decreases in blood pressure, the prevention of vascular complications, and recovery of insulin sensitivity and β -cell function [6].

The analytical methods, including high performance liquid chromatography (HPLC) with an ultraviolet detector [5,7] and LC-MS/MS [8,9] have been used in quantitative analysis of ipragliflozin in different biological matrices.

In this paper, we described validation of a method for accurate quantification of an ipragliflozin in bulk drugs and pharmaceutical dosage form along with validation as per ICH [10-12]. The plan of present study was to establish the inherent stability of ipragliflozin through stress studies under variety of conditions and develop a stability-indicating RP-HPLC method.

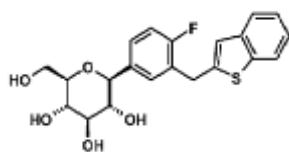


Fig. 1: Structure formula of ipragliflozin.

EXPERIMENTAL

Instruments

HPLC, constaMetric® 4100 LDC Analytical pump (Milton Roy, USA), equipped with SpectraSYSTEM UV3000 Diode-array UV-Visible detector and SpectraSYSTEM AS3000 auto sampler. The chromatographic analysis was carried out using (ChromQuest 4.2.34, version 3.1.6) data analysis program.

Samples

Ipragliflozine (99.8%) was kindly supplied by Al Andalous for Pharmaceutical Industries, Obour city, Egypt.

Chemicals and solvents

Acetonitrile and methanol, HPLC grade (Sigma-Aldrich, Germany)

Sodium hydroxide, hydrochloric acid and hydrogen peroxide, analytical grade (El-Nasr Pharmaceutical Chemicals Co., Egypt).

General procedure

Chromatographic conditions

A mobile phase system of methanol, acetonitrile and Water in ratio of (50: 25: 25, by volume) was used at a flow rate of 1 mL/min. The injection volume was 20 µL. The column used was HAMILTON column prp-x 100 C18 columns (15 cm X 4.6 mm, 5µm particle size). The eluents were monitored by the diode array detector (DAD) from 210 to 300 nm and peak area values were measured at 230 nm. All determinations were performed at ambient temperature.

Preparation of standard solutions and construction of calibration graph

Stock solutions (100 µg/mL) of ipragliflozin was prepared in methanol. The working standard solutions were prepared by dilution of aliquots of the stock solutions with methanol to reach the concentration ranges of 10-100 µg/mL. The stock and working solutions were kept away from light to prevent possible

photodegradation. Triplicate injections (20 μ L) were made for each concentration and analyzed under the previously described chromatographic conditions. Peak areas at 230 nm were plotted against the corresponding concentrations to construct the calibration graph and the linear regression equations was derived.

Assay of tablets dosage form

The content of seven Suglat[®] Tablets (50 mg of ipragliflozin per tablets) were mixed and weighed, after removing the film coated by scratching and washing with methanol, then a quantity equivalent to 10 mg of ipragliflozin was accurately transferred to 100-mL volumetric flask and the volume was made up to 75 mL with methanol. The solution was shaken vigorously for 15 min then sonicated for 30 min and completed to 100 mL with the same solvent to obtain a solution labeled to contain 100 μ g/mL of ipragliflozin. The solution was filtered with 0.45 μ m membrane filter then necessary dilutions of the filtrate were made with methanol to obtain different concentrations of ipragliflozin. The chromatographic conditions were applied for the prepared solutions and ipragliflozin content of Tablets was calculated using the corresponding regression equation.

Preparation of forced degradation solutions

Stock standard solution (100 μ g/mL) was used during forced degradation studies, and each degraded sample with a concentration of 80 μ g/mL was prepared in methanol. The prepared solutions were kept away from light to prevent possible photodegradation. Then, the procedure described under chromatographic conditions was followed. From the peak area of ipragliflozin in each chromatographed sample, the % degradation was then calculated. In each sample two mL of ipragliflozin stock standard solution was added to 10-ml volumetric flask.

- Acidic and basic hydrolysis were carried out using 2 mL of 0.1 N HCl, and 0.1 N NaOH for 12 and 24 hours at room temperature, respectively. Each sample was neutralized with alkali or acid before dilution.
- Neutral hydrolysis was done using 2 ml deionized water for 5 hours at 80 $^{\circ}$ C.
- Oxidative degradation was carried out using 1 mL 10 % H₂O₂ for 12 hours at room temperature.
- Photolytic study was done by exposing solutions of the drug to sunlight for 24 hours.
- Thermal study was carried out for 12 hours at 80 $^{\circ}$ C.

After the previous treatments, all forced degradation solutions were filtered with 0.45 μ m membrane filters prior to injection to the column.

RESULTS AND DISCUSSION

To date, no stability-indicating method was found in the literature for the determination of ipragliflozin. In this manuscript, the goal was to develop a new stability-indicating HPLC-DAD method for the determination of ipragliflozin in the presence of its possible degradation products.

Method development

A liquid chromatographic method coupled with diode array detection was developed to provide a suitable procedure for the stability study of ipragliflozin. The most important goal in liquid chromatographic method optimization is the achievement of sufficient resolution of the analytes with acceptable peak symmetry in a reasonable analysis time. The developed method was carefully designed and optimized to separate the cited compound from its forced degradation products.

To achieve this goal, three parameters should be optimized carefully. The first parameter is the reasonable choice of mobile phase. Several experiments were carried out using methanol, water and acetonitrile solutions in different ratios. It is noteworthy that ipragliflozin is relatively more hydrophobic with molecular weight of 404.45 which is considered a macromolecule. Using combinations of water and methanol increase the retention time of ipragliflozin. It has been noted that using water in small ratios with acetonitrile lead to

increase in the retention time with good resolution from its degradation products. By using mobile phase composition of methanol: acetonitrile : Water in ratio of (50: 25 : 25, by volume) a good separation was achieved with acceptable peak symmetry in a reasonable analysis time. The second parameter is the stationary phase selection. Different stationary phases were also tried such as BDS Hypersil C₁₈ column, Discovery[®] HS C₁₈ column and HAMILTON column prp-x100 C₁₈ column, these stationary phases gave near the results. The third parameter is the choice of the optimum wavelength at which the determination of ipragliflozin was done. The diode array detector allows the determination of ipragliflozin at different wavelengths simultaneously. Hence several wavelengths were tried but 230 nm which is the λ_{max} of ipragliflozin was chosen as this wavelength gave the best results regarding accuracy and sensitivity. The optimized chromatographic conditions showed a symmetric peak with minimum tailing and a reasonable analysis time. Figure 2 shows a typical chromatogram for ipragliflozin with retention time of 3.5 \pm 0.05 min. For more confirmation of the separation process the UV absorption curve of ipragliflozin have been recorded and it shows typical matching with the curve extracted from the DAD as shown in (Fig. 3).

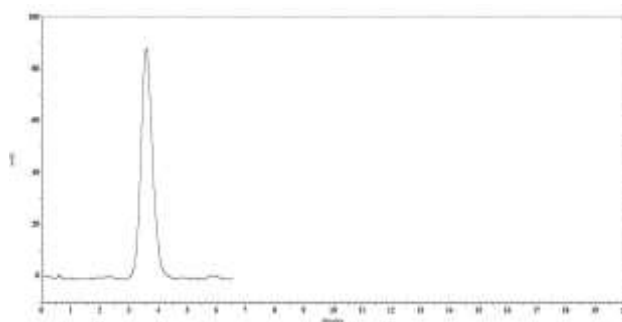


Fig. 2: HPLC chromatograms of 80 μ g/mL ipragliflozin at 230 nm

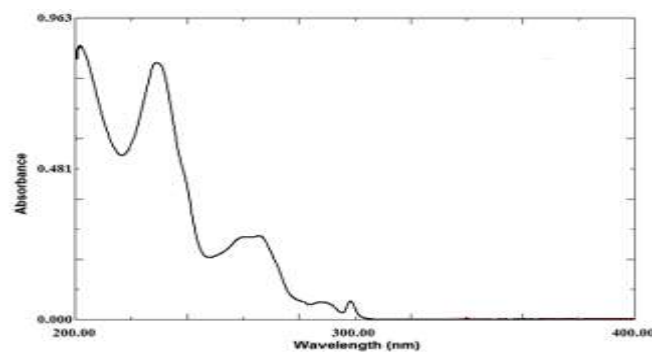


Fig. 3: The zero order UV absorption curve of 12 μ g/mL of ipragliflozin.

Forced degradation studies

Forced degradation studies were carried out on ipragliflozin to evaluate its degradation behavior by monitoring the chromatograms of ipragliflozin degradation products using the developed method. Due to the recent launch of this class of a novel class of hypoglycemic agents, different stress conditions were applied to evaluate the stability of ipragliflozin. It should be noted that all the recorded spectra during peak elution have been monitored using the diode array detector to detect any degradation present in the samples over the entire scanning area and to check the purity of the eluted peaks.

Ipragliflozin was found to be sensitive to hydrolysis by 0.1 N HCL for 24 hour at room temperature with appearing of one degradation product at 1.82 min (Fig. 4). Basic hydrolysis with 0.1 N NaOH for 24 hours at room temperature with appearing of one degradation product at 1.51 min (Fig. 5). Neutral hydrolysis was tried using deionized water for 12 and 24 hours at room temperature and at 80 $^{\circ}$ C and no degradation products were found.

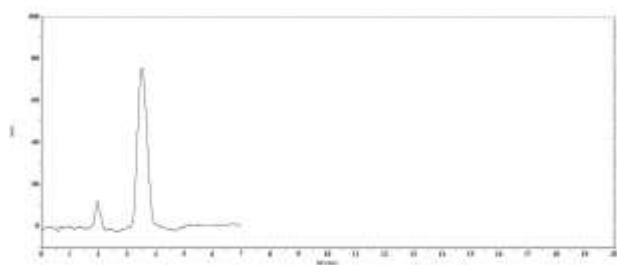


Fig. 4: HPLC chromatogram of ipragliflozin and its acidic degradation product using 0.1 N HCl for 24 hour at room temperature.

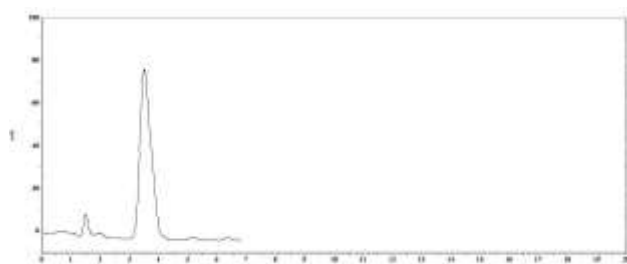


Fig. 5: HPLC chromatogram of ipragliflozin and its basic degradation product using 0.1 N NaOH for 24 hours at room temperature.

Oxidative degradation was tested using 10 % H₂O₂ for 12 hours. Samples were evaporated to expel the remaining H₂O₂. No degradation products were formed after 6 hour. However, after 12 hours one degradation product at 1.72 min was formed with complete resolving of ipragliflozin peak (Fig. 6). Great attention has been considered for the photo degradation study of ipragliflozin. The sample of ipragliflozin was exposed to sunlight for 6, 12 and 24 hours. No degradation products were formed and no reduction in peak area of ipragliflozin was observed after 6, 12 and 24 hours. Ipragliflozin was found to be thermally stable, as no additional peaks were observed when the drug was subjected to 80°C for 12 and 24 hours. The results of ipragliflozin stability studies are given in (Table 1).

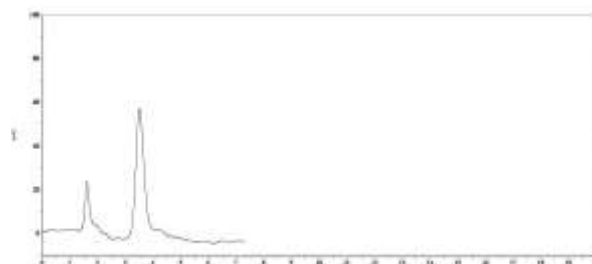


Fig. 6: HPLC chromatogram of ipragliflozin and its oxidative degradation product using 10 % H₂O₂ for 12 hours at room temperature

Table 1: Summary of Forced Degradation Studies.

| Stress conditions | Number of degradates (t _R) | % Degradation |
|---|--|---------------|
| 0.1 N HCL at room temperature for 24 hours | 1 (1.82) | 12.21 |
| 0.1 N NaOH at room temperature for 24 hours | 1 (1.51) | 9.43 |
| Neutral hydrolytic degradation at 80 °C for 24 hours | 0 | 0 |
| 10 % H ₂ O ₂ at room temperature for 12 hours | 1 (1.72) | 24.5 |
| Sunlight for 24 hours | 0 | 0 |
| Thermal at 80 °C for 24 hours | 0 | 0 |

System suitability

System suitability was checked by calculating different parameters including number of theoretical plates, Height equivalent to a theoretical plate, retention factor, resolution and tailing factor. The obtained values were found to be in the acceptable ranges when compared to USP reference values [13] as shown in (Table 2)

Table 2: System suitability results for the determination of ipragliflozin by the proposed HPLC method:

| Parameters | Ipragliflozin | Reference value [14] |
|---|---|--|
| Retention time (t _R) | 3.5 ± 0.05 | --- |
| Retention factor (K') | 1.21 | 1-10 |
| Theoretical Plates (N) | 2338 | >2000 |
| Height equivalent to theoretical plates (H in cm) | 0.011 | The smaller the value the higher the column efficiency |
| Tailing factor (T) | 1.201 | < 2 |
| Injection precision (%RSD) | 0.583 | < 1 |
| Resolution (Rs) | 0.1 N HCL (24 hour) | 1.82 |
| | 0.1 N NaOH (24 hours) | 1.51 |
| | 10 % H ₂ O ₂ (12 hours) | 1.72 |
| | | > 1 |

Method validation

The proposed HPLC-DAD method was validated according to the USP on validation of analytical procedures [13]. The linearity of the developed method was determined and the linear regression data for the calibration curve showed good linearity (r² = 0.9999) over

the concentration range of 10–100 µg/mL. The accuracy of the method was calculated by measuring the mean percent recovery of three determinations for three concentration of pure ipragliflozin and it was found to be 100.12. The precision of the developed method was checked by measuring % relative standard deviation (%RSD) of three concentrations for ipragliflozin repeated three times in the same day (repeatability) and in three days (intermediate precision). The obtained values of %RSD were < 2%, confirming the high precision of the developed method.

The specificity of the developed method was assessed by resolving ipragliflozin from its possible degradation products. The results revealed that the proposed method was able to completely discriminate ipragliflozin from all of its degradation products, confirming the specificity of the method. Moreover, the peak purity was checked using DAD and the purity of ipragliflozin was found to be more than 0.992 indicating that no additional peaks were co-eluted with the main compound.

Robustness of the developed method was studied by making small changes in flow rate (±0.1 mL/min) and the working wavelengths (± 2 nm). These variations did not have any significant effects on the peak area or retention time of ipragliflozin and %RSD of the responses were < 2% confirms the robustness of the method. The summary of these parameters and other validation and regression parameters are shown in (Table 3).

Application of the developed method

The developed method was applied for the assay of the cited drug in its commercial pharmaceutical formulation (Suglat® Tablets). No interfering peaks were observed from any of the excipients that may be present in the assayed preparation. The diode array detection enables peak purity verification where no signs of co-elution from any of the excipients were detected.

The standard addition technique was applied, where pure ipragliflozin was added to already analyzed pharmaceutical preparation. Good recovery of the pure standard was obtained confirming that the proposed method could be adopted for the

determination ipragliflozin in pharmaceutical dosage form without any possible interference from excipient or additives. The obtained results are shown in (Table 4).

Table 3: Validation and regression parameters of ipragliflozin by the proposed hplc method.

| Parameters | Proposed method | HPLC |
|--|------------------------|--------------------|
| Wavelength (nm) | 230 | |
| Linearity range (µg/mL) | 10 – 100 | |
| - Regression Equation | $y^a = b x^b + a$ | |
| - Slope (b) | 29636 | |
| - Intercept (a) | 1394.9 | |
| Coefficient of determination (r ²) | 0.9999 | |
| Accuracy | 100.61 | |
| Precision (%RSD) | Repeatability | 1.063 |
| | Intermediate precision | 1.180 |
| Robustness (%RSD) | | 0.749 ^c |
| | | 1.449 ^d |
| LOD (µg/mL) | 0.3508 | |
| LOQ (µg/mL) | 1.1694 | |

^a Peak area of ipragliflozin. ^b Concentration of ipragliflozin. ^c Robustness was checked by varying the flow rate (± 0.1 mL/min); ^d Robustness was checked by varying the wavelength of detection (± 2 nm).

Table 4: Standard addition technique of the proposed HPLC method.

| Pharmaceutical taken (µg/mL) | Pharmaceutical found (µg/mL) | Pure added (µg/mL) | Pure found (µg/mL) |
|------------------------------|------------------------------|--------------------|--------------------|
| 10 | 10.05 | 10 | 9.984 |
| | | 40 | 40.125 |
| | | 80 | 80.170 |
| Mean | | | 100.12 |

The obtained results of the developed method compared with the manufacturing method for determination of ipragliflozin by HPLC using Agilent TC (C18) column by isocratic elution of mobile phase with flow rate of 1.5 ml/min. The mobile phase composition was methanol -acetonitrile - phosphate buffer pH=3 (60:20:20, by volume). The eluate was monitored at 230 nm [14] are shown in (Table 5). The calculated *t* and *F* values are less than the tabulated ones indicating that there are no significant differences between the proposed and the reported method within a probability of 95% between the two methods.

Table 5: Determination of ipragliflozin in suglat® tablets by the proposed HPLC method and the reported method.

| Parameters | Proposed method | Reported method ^a (14) |
|--------------------------|----------------------------|-----------------------------------|
| Mean | 100.10 | 99.58 |
| %RSD | 0.391 | 0.299 |
| Number of measurements | 5 | 5 |
| Student's <i>t</i> -test | 0.455 (2.306) ^b | --- |
| <i>F</i> -value | 1.224 (6.388) ^b | --- |

^a The manufacturing method for determination of ipragliflozin by HPLC using Agilent TC (C18) column by isocratic elution of mobile phase with flow rate of 1.5 ml/min. The mobile phase composition was methanol -acetonitrile - phosphate buffer pH=3 (60:20:20, by volume). flow rate (1ml/min) and UV-detection at 230 nm.; ^b The values in parenthesis are the tabulated values of "*t*" and "*F*" at (P = 0.05)

CONCLUSION

This study described a simple, selective and reliable HPLC-DAD method for the estimation of ipragliflozin. To date, no comprehensive stability indicating assay method was developed for this drug by any analytical methodology. This work focused on the stability of the studied compound and its degradation behavior in different stress conditions. Significant advantages over the previously published literature were the simple mobile phase and

the minimum sample preparation required for the assay of ipragliflozin . The method was validated according to the USP recommendation and can be used for the routine analysis and for checking quality during stability studies of pharmaceutical preparations containing the cited compound.

Conflict of interest

The authors declare no conflict of interest.

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