Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Spectrophotometric Processing Datafor Determination Ipragliflozine in Pure and in Tablet Dosage Form

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ARTICLE HISTORY

Received: 16.04.2018

Accepted: 22.06.2018

Available online: 30.06.2018

Keywords:

Ipragliflozin; Degradation product.; Absorption factor; Firstdrevitave; Ratio

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ABSTRACT

Objective:This study aimed to develop three simple UV spectrophotometric methods for determination of ipragliflozin in bulk drugs and pharmaceutical dosage form in the presence of its oxidative degradation product.Methods:Manipulating ratio spectra were developed for the interested methods. The first method is an absorption factorusing Zero-order absorption spectraof intact and its oxidative degradation product,the peak amplitudes of method were measured at 229 nm.Thesecond method isthe firstdrevitave using zero-order absorption spectra for derivatization and the peak amplitudes of method were measured at 257.8 nm. The third method isthe ratio subtraction using 14 µg/mL of its oxidative degradation product as a divisor, finally the peak amplitudes of methodwere measured at 229 nm..The results and discussion:The calibration curve is linear over the concentration range of 2-14 µg/mL, the proposed methods were validated according to International Conference on Harmonization (ICH) guidelines and successfully applied for the determination of ipragliflozinin the presence of its oxidative degradation product.Conclusion:The proposed three methods are simple, rapid, economical, accurate and precise for determination of ipragliflozinin the presence of its oxidative 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 [14]. 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; there-fore, SGLT2 inhibitors may reduce blood glucose levels and body weight [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].

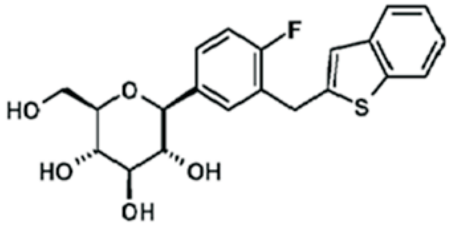

Fig. 1 : Structure formula of ipragliflozin.

The analytical methods, including high performance liquid chromatography (HPLC) with an ultraviolet detector [5,8] and LCMS/MS [9,10] 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 [11-12]. 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.

Instrument:

Shimadzu UV/visible, double beam 1800 Spectrophotometer (Tokyo, Japan) with 1 cm matched quartz cells and UV Probe software (2.35 ver.).

MATERIALS AND METHODS

Materials:

- Ipragliflozine (99.8%) was kindly supplied by Al Andalous for Pharmaceutical Industries, Obour city, Egypt.
- **Pharmaceutical preparation:** Suglat® tablets, was manufactured in Japan and was kindly supplied by Al Andalous for Pharmaceutical Industries, Obour city, Egypt.
- Methanol, analytical grade (El-Nasr Company, Egypt).

Preparation of standard solutions:

Standard solutions (100 µg /mL) of ipragliflozinand its oxidative degradation productwere prepared by transferring accurately weighed 10 mg of standard drugs into two separate 100-mL volumetric flasks, then dissolved in methanol and completed to the mark with the same solvent.

Construction of calibration curves (linearity):

Accurately measured aliquots equivalent to (0.20 □ 1.40 mL)of each standard were transferred from their standard solutions (100 µg/mL) into two separate series of 10-mL volumetric flasks and the volume of each flask was diluted up to the mark with methanol, to reach the concentration range of (2 □ 14µg/mL). The absorption spectra of these solutions were measured in the range of 210 to 320 nm against methanol as a blank.

Laboratory prepared mixtures:

Accurate aliquots equivalent to (20-120 µg) of ipragliflozininto a series of 10 ml volumetric flasks from its standard solution (100 µg/mL) and portion equivalent to (120 20 µg) of its degradation productfrom its standard solution (100 µg/mL) were added to the same flasks and volumes were completed to mark with pure methanol and mixed well.

Absorption factormethod [13]:

The absorption spectra (from 210 to 320 nm) of these solutions were recorded using methanol as a blank. The average value of absorption factor of its degradation product(abs 229 nm/abs 310 nm) was calculated. The absorbance value of ipragliflozinat 229 nm were determined from equation[13]which corresponding to concentrations of ipragliflozin.

First derivative [14]:

Zero-order absorption spectraofipragliflozinand its degradation product.First - derivative (^{1}D) spectra of the drug were recorded against methanol as blank. The amplitude of the trough at 257.8 nm was measured for each drug concentration.

A Calibration curve relating trough amplitude to drug concentration in µg ml^{-1} was constructed, the regression equation was derived.

Ratio subtraction method [15]:

The absorption spectra of ipragliflozinwere divided by the spectrum of 14µg/mL of its degradation product(divisor) to give ratio spectra, then the constant was determined from the plateau region, then subtracted from the ratio spectra, the original spectra of ipragliflozinwas obtained after the multiplication of the obtained spectra by the spectrum of the divisor. The absorbance at 229 nm (λmax of ipragliflozin) was determined against the corresponding concentration of ipragliflozinto give the corresponding regression equation.

Application to pharmaceutical formulation:

The content of seven Suglat® Tablets (50 mg of ipragliflozin per tablets) were mixed and weighed, after removeing the film coated by scratching and washing with methanol. An amount of the powder equivalent to 10 mg of ipragliflozinwas weighed, dissolved in methanol by shaking for about 30 min. The solution was filtered and transferred quantitatively into 100-mL volumetric flask. The filtration system was evaluated to ensure that the filter does not adsorb the drug. The volume was then completed to the mark with methanol. Necessary dilutions were made to reach concentrations in the linearity range. The same procedures under the corresponding linearity were applied and the concentrations of ipragliflozin was calculated from the corresponding regression equations.

RESULTS

Simple spectrophotometric methods were developed for the determination of ipragliflozininthe presence of its degradation productwithout previous separation.

The absorption spectra of the resulting solution of ipragliflozinandits degradation productwhich containing (2-14µg/mL) were measured and stored in the computer. Where,

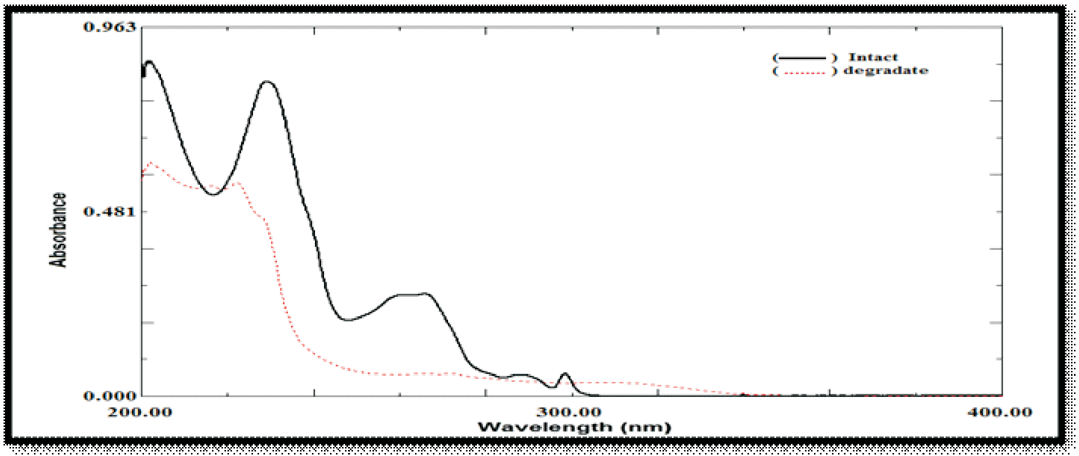

Fig. 2 : Zero order absorption spectra of ipragliflozin (12µg/mL) and its oxidatative degradation product(12µg/mL).

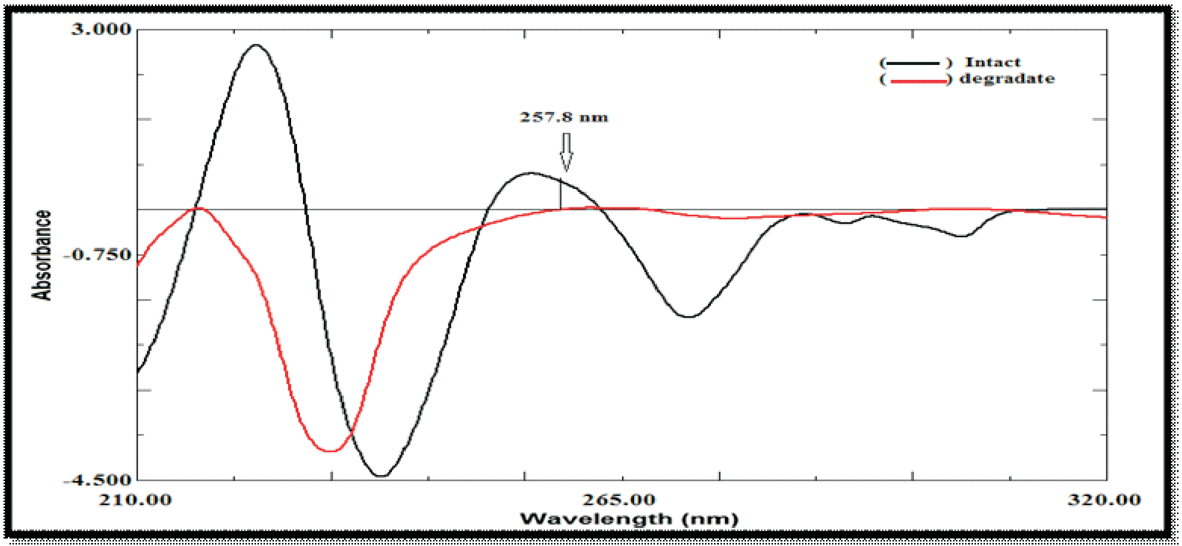

Fig. 3 : First derivative of absorption spectra of Intact (12 μg ml^{-1}) and its Degradation Product (12 μg ml^{-1}) in methanol.

these absorption spectra of both show severe overlapping and its degradation productspectrum is extended more than ipragliflozinspectrum (Figure 2).

1- Absorption factor method:

Quantitative estimation of X in mixture (X+ Y) was carried out by subtracting the absorption duetoYat$^{\lambda}_{max}$ofXusing experimentally calculated absorption factor.

Absorption ofXat$^{\lambda}_{1}$=abs$^{\lambda}_{1}$ (X +Y) {abs1/ abs2}x{abs$^{\lambda}_{2}$ (X +Y)} (1)

Where; abs 1 and abs 2 are the absorbance of Y at$^{\lambda}_{1}$ and$_{2}$, (abs1/ abs2) is called the absorption factor and it is constant forpure Y, and abs$^{\lambda}_{1}$ (X+Y)andabs$^{\lambda}_{2}$ (X +Y) are the absorption of the mixtureat these wavelengths. The concentration of X is calculated from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves (Figure 2) ofXatits$^{\lambda}_{max}$ against the corresponding concentrations).

X= ipragliflozin, Y= its oxidative degradation product

2- Firstderivative method:

It is clear from the spectra in (Fig 2) that, there is a band overlapping between the drug and its degradation product. Such overlapping was eliminated by the first derivative (^{1}D) scanning of ipragliflozinand its degradation product in methanol, ipragliflozinhas a trough at 257.8 nm which shows no interference from the degradation product. Thus it would be possible to adopt the (^{1}D) spectrophotometry at 257.8 nm for direct determination of ipragliflozinin presence of its degradation product as seen in (Fig 3).

1- Ratio subtraction method:

A mixture of two components X and Y with overlapping spectra can be resolved by ratio subtraction if the spectrum of component Y is extended more than X. Component X can be determined by dividing the spectrum of mixture by certain concentration of Y as a divisor (Y').

The will give a new curve that is represented by:

X / Y' + constant

If the consultant determined directly from the spectra and subtracted, the new spectrum multiplied by Y', the original spectrum of X is obtained.

This can be summarized in the following equation:

(X+Y) / Y' = (X / Y') + (Y / Y') (1)

(X+Y) / Y' = (X / Y') + Constant (2)

By subtraction of the constant from equation (2):

X / Y' + Constant Constant = X / Y' (3)

By multiplication of equation (3) by a divisor (Y'):

As a result, (X / Y') × Y' = X (4)

The constant can be determined directly from the spectrum (X+Y) / Y' by the straight line which is parallel to the wavelength axis in the region since Y is extended.

The extended zero absorption spectrum of ipragliflozinthan zero absorption spectrum of its degradation productis necessary for application of this method as shown in (Figure 2). Ratio spectra of laboratory prepared intact (2-14 μg ml^{-1}) using 14 μg ml^{-1} of degradate as a divisor(Figure 4). Ratio spectra of laboratory prepared of intact (2-14 μg ml^{-1}) using 14 μg ml^{-1} of degradate as a divisor after subtraction of the constant (Figure 5), The zero-order absorption spectra of intact (2-14 μg ml^{-1}) obtained by the proposed ratio subtraction method for the analysis after multiplication by the divisor(Figure 6).

DISCUSSION

Good linearity is obtained in the concentration range of (2□ 14μg/mL) for three methodes. The corresponding regression equations was computed asin (table 1)

Validation of the method:

The proposed methodwastestedinthe linearity range, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision according to International Conference on Harmonization (ICH) guidelines [11-12].

Linearity and range:

According to the theory of all methods, the linearity of

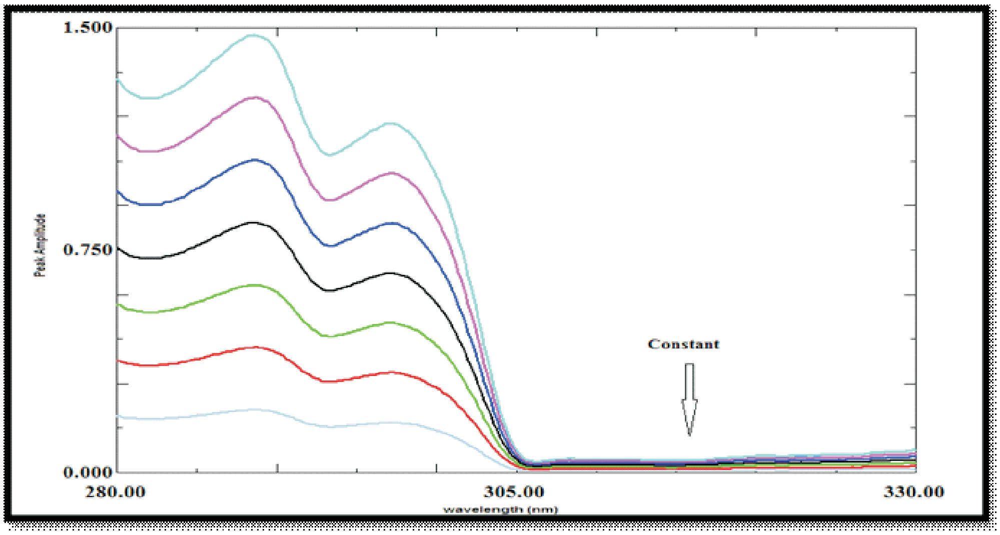

Fig. 4 : Ratio spectra of laboratory prepared intact (2-14 µg ml^{-1}) using 14 µg ml^{-1} of degradate as a divisor.

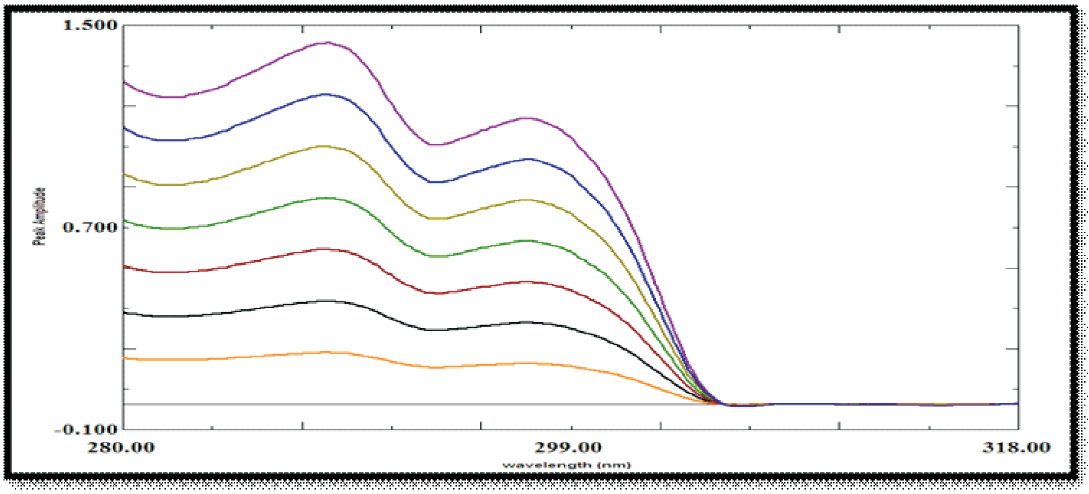

Fig. 5 : Ratio spectra of laboratory prepared of intact (2-14 µg ml^{-1}) using 14 µg ml^{-1} of degradate as a divisor after subtraction of the constant.

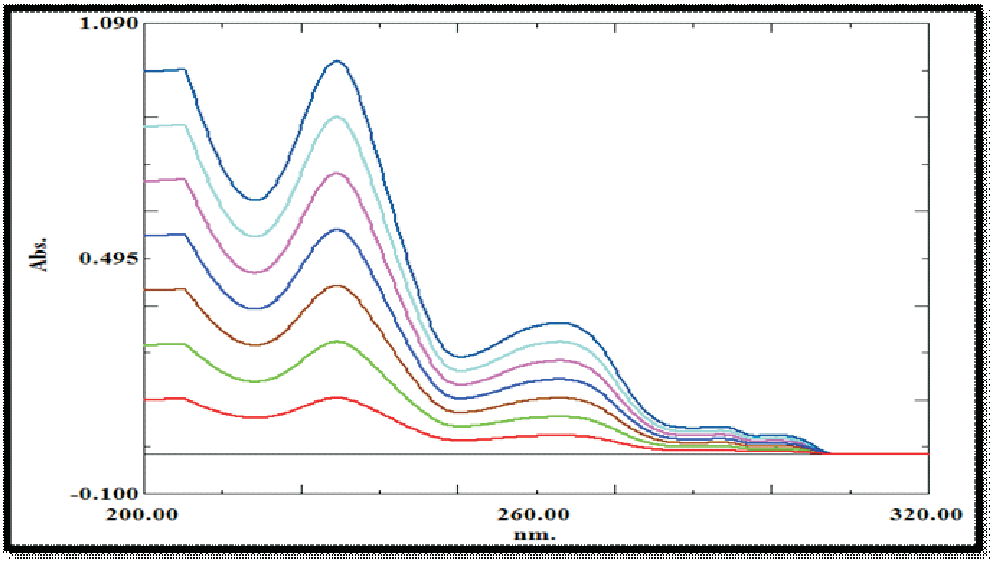

Fig. 6 : The zero-order absorption spectra of intact (2-14 µg ml-1) obtained by the proposed ratio subtraction method for the analysis after multiplication by the divisor.

Table 1 : Spectral and validation data for determination of ipragliflozinin presence of its oxidative degradation product by the proposed methods:.

Parameters	**Absorption factor**	**First derivative**	**Ratio subtraction**
Wavelength (nm)	229 and 310	257.8	229
Linearity range (µg/mL)	2 — 14	2 — 14	2 — 14
LOD (µg/mL)	0.173	0.190	0.216
LOQ (µg/mL)	0.525	0.575	0.654
Regression equation			
Slope	0.0672	0.0399	0.0706
Intercept	0.0193	0.004	0.0004
Correlation coefficient (r)	0.9999	0.9998	0.9998
Accuracy (%R)*	99.53	99.76	100.47
Precision (%RSD)*			
- Repeatability (intra-day)			
- Intermediate precision	0.941	0.895	1.152
(inter day)	1.008	0.976	1.287

* Average of three determinations of three concentration levels.

ipragliflozinwas determined by measuring the peak amplitudes of spectra after compelet manipulate at different wavelength related to methods as in (table 1)

The calibration graphs for all proposed methods were rectilinear over the concentration range of 2-14µg/mL for ipragliflozinwith high values of the correlation coefficient, the data is supplied in (table 1)

Limit of detection (LOD) and limit of quantification (LOQ):

LOD and LOQ were calculated according to to ICH guidelines [11-12] from the following equations:

LOD = 3.3 Sa / slope

LOQ = 10 Sa / slope

Where Sa is the residual standard deviation of a regression line.

LOD and LOQ values of ipragliflozinfor each method were listed in (table 1)

Selectivity:

The selectivity of the methods was achieved by the analysis of different laboratory prepared mixtures of ipragliflozin and its oxidative degradation product within the linearity range. Satisfactory results listed in Table 2, and the results of the standard addition technique Table 3 prove that the proposed methods can selectively analyze ipragliflozin without any interference from its degradation productor the excipients.

Accuracy:

Accuracy of the proposed methods, calculated as the mean percent recovery (%R), was assessed by applying the proposed procedures for triplicate determination of three concentration levels covering the specified range for drug (2, 6 and 12µg/mL). The concentrations were obtained from the corresponding regression equations and the mean percent recoveries, shown in Table 1, indicate the accuracy of the proposed methods. Accuracy of the methods was further assured by the use of the standard addition technique. It was performed by the addition of known amounts of pure ipragliflozinandits degradation productto known concentrations of the pharmaceutical preparation were assayed, and the results obtained were compared with the expected results (Table 3). The good recoveries of the pure added ipragliflozin suggested good accuracy of the proposed methods.

Precision:

The precision of the proposed methods, calculated as percent relative standard deviation (%RSD) of the percent recoveries, was checked by applying the proposed procedures for triplicate determination of three concentration levels covering the specified range for drug (2, 6 and 12µg/mL) in the same day (intra-day analysis) for repeatability and on three different days (inter day analysis) for intermediate precision. The results in Table 1 indicate precision of the method.

Application to pharmaceutical formulation:

The proposed methods were applied for the determination of ipragliflozinin their pharmaceutical formulation, Suglat® Tablets (50 mg of ipragliflozin per tablets). Satisfactory results were obtained in good agreement with the label claim, and the results of the standard addition technique indicate no interference from excipients and additives (Table 3).

Table 2 : Determination of ipragliflozininthe presence of its oxidative degradation productin the laboratory prepared mixtures by the proposed methods:

Method	Concentration taken (μg/mL)		Concentration found (μg/mL)	% Recovery
	Ipraglifloz.	Deg.	ipragliflozin	ipragliflozin
Absorbtion factor	2	12	1.96	98.19
	4	10	4.04	101.08
	7	7	7.03	100.48
	10	4	9.81	98.13
	12	2	12.21	101.77
	Mean ± %RSD			**99.93±1.681**
First dervative	2	12	2.03	101.50
	4	10	4.02	100.50
	7	7	6.97	99.57
	10	4	10.07	100.70
	12	2	11.95	99.58
	Mean ± %RSD			**100.37±0.812**
Ratio Subtraction	2	12	1.99	99.58
	4	10	3.94	98.65
	7	7	7.09	101.30
	10	4	10.04	100.37
	12	2	12.10	100.87
	Mean ± %RSD			**100.15±1.053**

Table 3 : Determination of ipragliflozinin Suglat® Tablets by the proposed methods and application of standard addition technique:

Proposed methods	Suglat® Tablets		Standard addition technique			
	Ipragliflоz content* (μg/mL)	% Found* ± %RSD	Taken (μg/mL)	Pure added (μg/mL)	Pure found* (μg/mL)	% Recovery
	ipragliflozin		Ipraglifloz.	Ipraglifloz.	Ipraglifloz.	Ipraglifloz.
Absorbtion factor	4.02	**100.15 ± 0.434**	4	4	3.97	99.25
	5.98			6	6.03	100.50
	10.03			10	9.96	99.60
	Mean ± %RSD					**99.78 ± 0.646**
First dervative	3.96	**99.26 ± 0.312**	4	4	4.06	101.5
	5.95			6	6.13	102.17
	9.96			10	9.94	99.4
	Mean ± %RSD					**101.02± 1.429**
Ratio Subtraction	3.93	**99.38 ± 1.132**	4	4	4.03	100.75
	6.03			6	6.07	101.17
	9.94			10	10.06	100.60
	Mean ± %RSD					**100.83±0.291**

* Average of three determinations

Table 5 : Statistical comparison between the results obtained by the proposed methods and the manufacturing method for the determination of ipragliflozin[16] in pure powder form:

Parameters	Absorbtion factor	First dervative	Ratio subtraction	Manufacturing method[16]
	ipragliflozin.	ipragliflozin	ipragliflozin	ipragliflozin
n	5	5	5	5
Mean %R	99.88	99.60	100.12	99.58
SD	1.032	1.366	0.927	1.140
%RSD	1.033	1.371	0.925	1.144
Student's t-test (2.306)(c)	1.220	1.435	1.512	——
F value (6.388)(c)	1.295	2.101	1.979	——

(c) The values in the parenthesis are tabulated values of t and F at (p= 0.05).

Statistical analysis:

Showed statistical comparison of the results obtained by the proposed methods and the manfuacturing method [16] in their pure form in Table 4. The calculated t and F values were less than the theoretical ones indicating that; there was no significant difference between the proposed and the reported methods with respect to accuracy and precision.

CONCLUSION

Although these proposed methodsarenot advanced, but their values arise from the lack of any published method for determination of ipragliflozinin the presence of PGZ in their new combination.

The proposed methods are simple, rapid, economical, accurate and precise and can be used for determination of ipragliflozinin the presence ofits oxidative degradation productin pure form as well as in pharmaceutical dosage form without interference from excipient and no need for previous separation steps.

ACKNOWLEDGMENT

I am deeply thankful to ALLAH, by the grace of whom this work was realized. I wish to express my indebtedness and gratitude to staff members of the Analytical Chemistry Department for their valuable supervision, continuous guidance, and encouragement throughout the whole work.

Conflict of interest:

The authors declare no conflict of interest.

REFERENCE

1. Kadokura T, Zhang W, Krauwinkel W, Leeflang S, Keirns J, Taniuchi Y, Nakajo I, Smulder R. Clinical Pharmacokinetics and Pharmacodynamics of the Novel SGLT2 Inhibitor Ipragliflozin. Clin. Pharmacokinet. 2014:53, 975988.
2. Imamura M, Nakanishi K, Suzuki T, Ikegai K, Shiraki R, Ogiyama T, Tomiyama H. Discovery of Ipragliflozin (ASP1941): a novel C-glucoside with benzothiophene structure as a potent and selective sodium glucose co-transporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes mellitus. Med. Chem. 2012: 20, 32633279.
3. Schwartz SL, Akinlade B, Klasen S, Kowalski D, Zhang W. Pharmacokinetic, and Pharmacodynamic Profiles of Ipragliflozin (ASP1941), a Novel and Selective Inhibitor of Sodium-Dependent Glucose Co-Transporter 2, in Patients with Type 2 Diabetes Mellitus. Diabetes Technol. Ther. 2011: 13, 12191227.
4. Veltkamp SA, Kadokura T, Krauwinkel WJ, Smulders RA. a novel selective sodium-dependent glucose co-transporter 2 inhibitor, on urinary glucose excretion in healthy subjects.Clin. DrugInvestig. 2011: 31, 839851.
5. Salama FM, Attia KA, Abouserie A, Mabrouk R, Abdelzaher AM. Stability-Indicating Hplc-Dad Method For The Determination Of Ipragliflozine. Innorig. Internat. J. of Sci., 2018: 5 (1), 11-15
6. Tahara A, Kurosaki E, Yokono M, Yamajuku D, Kihara R, Hayashizaki Y, Takasu T, Imamura M, Noda A. Effects of SGLT2 selective inhibitor ipragliflozin on hyperglycemia, hyperlipidemia, hepatic steatosis, oxidative stress, inflammation, and obesity in type 2 diabetic mice. Eur. J.Pharmacol. 2013: 715, 246255.
7. Kurosaki E, Ogasawara H. Ipragliflozin and other sodium-glucose cotransporter-2 (SGLT2) inhibitors in the treatment of type 2 diabetes. preclinical and clinical data. Pharmacol. Ther. 139 (2013) 5159.
8. Tahara A, Kurosaki E, Yokono M, Yamajuku D, Kihara R, Hayashizaki Y, Takasu T, Imamura M, Kobayashi Y, Noda A, Sasamata M, Shibasaki M. Pharmacological profile of ipragliflozin (ASP1941), a novel selective SGLT2 inhibitor, in vitro and in vivo. NaunynSchmiedebergs Arch. Pharmacol. 2012: 385, 423436.
9. Kadokura T, Akiyama N, Kashiwagi A, Utsuno A, Kazuta K, Yoshida S, Nagase I, Smulders R, Kageyama S. Pharmacokinetic and pharmacodynamic study of ipragliflozin in Japanese patients with type 2 diabetes

mellitus: A randomized, double-blind, placebo-controlled study. Diabetes Res. Clin. Pract. 106 (2014) 5056.

10. Kobuchi S, Ito Y, Yano K, Sakaeda T. A quantitative LCMS/MS method for determining ipragliflozin, a sodium-glucose co-transporter 2 (SGLT-2) inhibitor, and its application to a pharmacokinetic study in rats. Journal of Chromatography B 1000 (2015): 22-28.
11. International conference on harmonization (ICH) of technical requirements for the registration of pharmaceuticals for human use. Validation of analytical procedures methodology. ICH-Q2 (R1), Geneva1996, 1-8.
12. ICH Stability testing of new drug substance and product Q1A (R2), International conference on Harmonization, IFPMA, Geneva 2003.
13. Prajapati JP, Patel MB, Prajapati RJ andPrajapati NA. Simultaneous determination of perindopril erbumine and amlodipine besylate by absorption factor method. Int J ApplBiol Pharm Tech., 2011: 2 (3) 230233.
14. Khalid AM, Mohammed WN, Ahmed M. Five Spectrophotometric Methods For Determination Of Amisulpride In Pure Form And Pharmaceutical Dosage Form.Eur. J. Biomed. Pharm. Sci. 2015: 2 (3) 593-608.
15. Khalid AM, Mohamed WI, Ahmad AM, Ashraf A; Simple stability indicating ultraviolet spectroscopic methods for the estimation of ezetimibe in presence of its alkaline degradation product. ACAIJ, 2016: 16, 382-93.
16. Quality control and quality assurance unit; Al Andalous Pharmaceutical Industries Company, Cairo, Egypt.