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Simultaneous Determination and Stability Assessment of Metformin and Sitagliptin in Pharmaceutical Form by High Performance Liquid Chromatography

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ABSTRACT

A simple, rapid and highly selective chromatographic procedure is developed for simultaneous quantification of Metformin and Sitagliptinin tablet formulation. The active ingredients are separated using dihydrogen phosphate buffer (pH 6.00)/acetonitrile mobile phase, flow rate 1.0 mL.min-1, and with DAD detection at 218 nm. The stability and assay separation process are accomplished in 9.0 min with high resolution. The method is precise and accurate. A wide dynamic ranges (1.0-15.0) and (2.0-150.0) mg/L for Metformin and Sitagliptin, respectively, is employed. Both drugs are quantified down to 0.1 and 1.0 mg/L for Metformin and Sitagliptin, respectively, which indicates the high sensitivity of the procedure.

Finally, the stability-indicating capability of the procedure is accomplished for tablet formulation (50 mg Metformin and 500 Sitagliptin), and the results indicated that Metformin is unstable to UV and H2O2 with degradation higher than 30.0%. Furthermore, Sitagliptin is unstable at acidic, basic, and oxidation environments.

Keywords: Simultaneous Determination; Metformin; Sitagliptin; High Performance Liquid Chromatography; Stability Indicating.

1. INTRODUCTION

Metformin HCl (MET) is the short name for N,N-dimethyl imido dicarbonimidic diamide (Figure 1). It is an oral anti-diabetic drug in the biguanide class [(1)]. MET is the first line choice for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function. Evidence is also mounting for its efficacy in gestational diabetes, although safety concerns still preclude its widespread use in this setting. It is also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor[(2)].

Sitagliptin (SIT), is an oral anti hyperglycemic (anti-diabetic drug) of the DPP-4 inhibitor class [(3, 4)]. This enzyme-inhibiting drug is used either alone or in combination with other oral antihyperglycemic agents for treatment of diabetes mellitus type2[(5)]. The advantage of this medicine is its lower side effects (e.g., less hypoglycemia, less weight gain) in the control of blood glucose values. Sitagliptin was approved for marketing in the EU in 2007 and currently has therapeutic indications as

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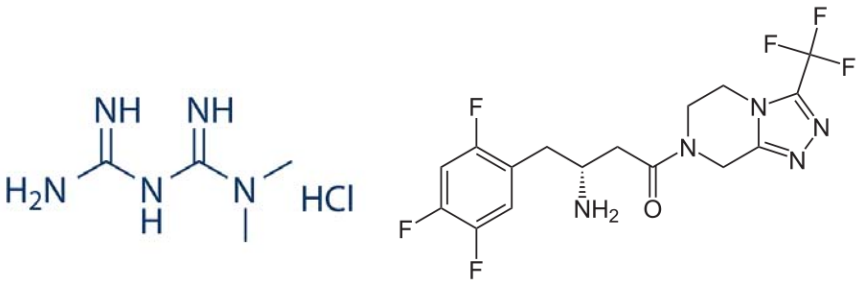
a second line treatment for patients with ***type 2 diabetes mellitus*** (T2DM) to be used in combination with Metformin, a PPAR-γ agonist, or a sulphonyl urea (alone or in combination with Metformin) [6].



Metformin HCl Sitagliptin

Figure 1: Chemical structure of the drugs

The combination of the two components, Metformin HCl and Sitagliptin (Figure 1), provides complementary mechanisms of action for lowering glucose. This leads to a new treatment option, which has the potential to cure patients with ***type 2 diabetes mellitus*** (T2DM). It is indicated as an adjunct to diet and exercise to improve glycaemic control in patients inadequately controlled on Metformin HCl alone. They improve blood glucose control more potently than either compound separately[7].

Several analytical methods have been reported in the literature for the quantification of Metformin in formulations as well as biological fluids, such as liquid chromatography used for simultaneous determination of Metformin and Pioglitazone in pharmaceutical dosage forms[8]. Sitagliptin was accurately quantified in pharmaceutical formulations and biological fluids using spectrophotometric method [9, 10]. Zeng used mass spectrometry (LC–MS/MS) to determine Sitagliptin in human plasma using protein precipitation and tandem mass spectrometry[11].

Limited studies analyze the stability and assay assessment of Sitagliptin and Metformin simultaneously. Ghazala estimated Metformin and Sitagliptin in tablet dosage form[12]. Attimarad studied the separation and stability of Metformin and Linagliptin on the tablet formation using RP-HPLC[13]. Spectrophotometric method based on multi-component mode of analysis at wavelength of 232 nm and 267 nm for Metformin and Sitagliptin respectively was employed[14].

In the present work, a new, selective, rapid and accurate method for the determination of Metformin and Sitagliptin which often dosage in variable levels (1:10 and 1:20 MET: SIT) is developed and validated. This method is combined pharmaceutical formulation, using a high performance liquid chromatography (HPLC). In addition, studying the ability of the developed method to serve as a high-impact tool for the simultaneous analysis of the combined actives in pharmaceutical tablet was conducted. The formulated drugs exposed initially into various vigorous forced degradation conditions (acidic, basic, heat, radiation, and oxidative). Forced degradations was applied, assessed the powerful and reliability of the present method to measure both drugs at different stability conditions.

2. Experimental

Reagents and Sample

Metformin HCl raw material, certified to contain 99.95% (USV Limited, India). Sitagliptin phosphate monohydrate, certified to contain 99.85% (Beijing Huikang, China). A formulated tablet nominally containing 500 mg of Metformin HCl and 50mg of

Sitagliptin phosphate monohydrate per tablet was purchased from a local pharmacy. The chemicals anhydrous potassium dihydrogen phosphate, HPLC grade triethylamine and acetonitrile were purchased from Merck chemical company(Merck, Germany).

Chromatographic system and conditions

Chromatographic separation was achieved on C8 column (25.0 cm x 4.6mm, 5.0 µm) supplied by ACE company, applying an isocratic elution based on potassium dihydrogen phosphate buffer-acetonitrile (70:30, v/v) as mobile phase at pH (6.00). Photodiode array (PDA) detector was operated at 218 nm wavelength. The mobile phase was pumped through the column at flow rate 1.0 mL/min. Analyses were performed at ambient temperature and the injection volume was 20 µL. The HPLC system used in this work consisted of a Shimadzu LC-2010C HT Liquid chromatography (Shimadzu, Japan), equipped with Photodiode array (PDA) SPD-M20A Detector. The potassium dihydrogen phosphate buffer (0.05M) was prepared by dissolving (6.8 g) of anhydrous salt in 1000mL of water. The mixture based on buffer-acetonitrile (70:30 v/v) as a mobile phase, adjusted to pH (6.00 + 0.05) by triethylamine base.

Preparation of standard solutions

A standard stock solution (1000.0 µg/mL) was prepared by dissolving 50.0 mg of Metformin in 50.0 mL mobile phase; the resulting solution was sonicated for 10.0 minutes. A 12.8 mg of Sitagliptin phosphate (equivalent to 10.0mg of Sitagliptin) with the addition of 1.0mL of standard stock solution was dissolved in 100.0mL mobile phase followed by sonication for 10.0 minutes. Analyses were carried out by using standard solution of 10.0 µg/mL Metformin and 100.0 µg/mL Sitagliptin.

Preparation of sample solutions

Twenty tablets were separately weighed and the average weight of each tablet (0.75 g) was calculated. Fine powdered tablets equivalent to 50 mg of Metformin and 5 mg of Sitagliptin was weighed and transferred quantitatively into 50.0 mL volumetric flask containing 20.0 mL of mobile phase, the solution was sonicated for 10.0 minutes and made up to the mark with mobile phase, resulted solution was filtered and used to analyze Sitagliptin in pharmaceutical tablet (sample stock solution 100.0 µg/ mL Sitagliptin). Aliquot of diluted sample solution was prepared by diluting 1.0 mL of (sample stock solution) in 100.0 mL with mobile phase; diluted sample solution was filtered and used to analyze Metformin pharmaceutical tablet form (sample diluted solution 10.0 µg/ mL Metformin).

3. Results and Discussion

To get better performance analysis for both drugs, the chromatographic parameters were adjusted to ensure complete separation in real samples and formulation. The following sections summarizes the analytical performance for the proposed method toward drugs separation at the optimum conditions.

Quality of chromatographic separation of drugs

It was necessary to assess the chromatographic behavior of the proposed procedure before running quantitative analysis for drugs. Accordingly, we estimate the stability parameters for both drugs at the best separation conditions.

The present work studies the detection of both drugs at different wavelengths (210, 220, 230, and 250 nm). wavelength at 218 nm gives a typical chromatogram for both drugs, Figure 2. It generates Noise-free base line with symmetric peaks. In fact, and the best detection was noted at 218 nm. Accordingly, all samples are measured at 218 nm.

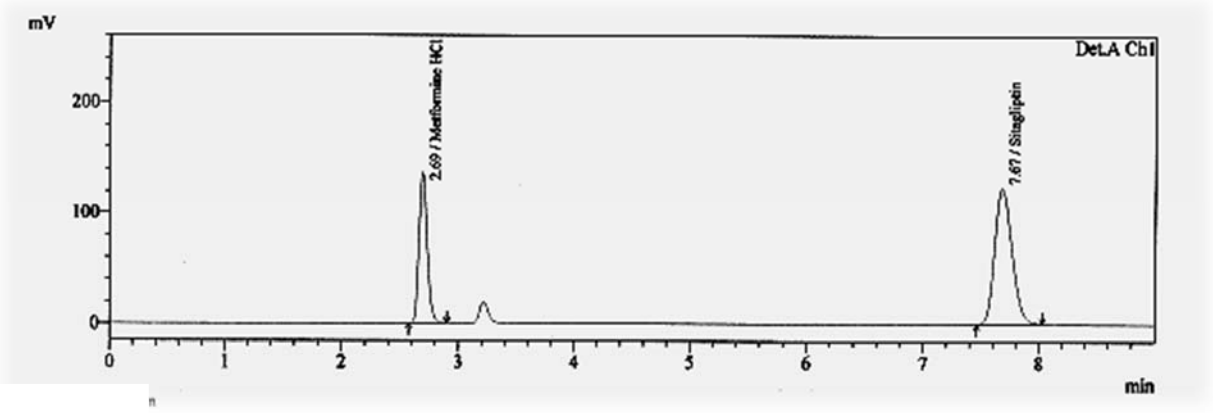


Figure 2: Typical chromatogram of MET and SIT at 218 nm

The quality of chromatographic separation of both drugs are assessed by estimating number of stability parameters for MET and SIT, Table 1.

Table 1. Stability parameters for the proposed chromatographic procedure*

Drug	Retention time	Peak width	Asymmetry	Tailing	Number of theatrical plates
Metformin	2.6	0.2	0.8	0.71	1,200
Sitagliptin	7.7	0.6	0.9	0.82	4,000

* The provided data were estimated from four identical chromatograms taken at 20.0 mg/L ppm for both drugs (n=4, RSD < 10.0%)

Table 1 indicates reasonable elution times of both drugs with a total run of 10.0 mints. The estimated resolution value is about 5 which indicates the complete separation of the bands. The reported run time is the optimum one under the applied conditions. The interesting point in Table 1 is the convenient shapes of the peaks with no serious peak forting and tailing. The reported asymmetric indices for both drugs Are with the typical range for symmetric peaks (0.4-1.8)[15]. The excellent chromatographic performance for the drugs separation is revealed from the large number of theoretical plates. The estimated number of plates is 1,200 and 4,000 for MET and SIT, respectively. SIT has wider peak width than MET and this indicates higher band diffusion and affinity toward the stationary phase. Based on the above discussion, the proposed chromatographic procedure is selective and sensitive for quantification of both drugs in their mixtures and also in the real formations after elimination of other excipients.

Figure of merit and quantitative determination of the binary-drug system

Figures of merit along with determination of the drugs in their synthetic mixture should be discussed. The analytical performance of the proposed method was further evaluated by running many chromatographic tests on binary solutions of drugs at different levels. Table 2 summarizes the final results.

Table 2. Analytical characteristics of the proposed chromatographic test for binary-drug system

Parameter	MET	SIT
Dynamic Range (mg/L)	1.0-15.0	2.0-150.0
Linearity	0.9993	0.9994
DL (mg/L)	0.03	0.3
QL (mg/L)	0.1	1.0
Intra-day precision (*n*=6, %RSD)	6.6	1.3
Inter-day precision (*n*=6, RSD)	8.3	2.7
Accuracy(%) *n*=6	99.0	101.3
Robustness (RSD)	2.5	1.5

Calibration curves for drugs are generated by plotting peak area versus concentration over the ranges 1.0-15.0 and 2.0-150.0 mg/L for MET and SIT respectively. The calibrations for both drugs are linear with R^2 higher than 0.999. The high linearity indicates the linear correlation between peak area and drug concentration. The generated calibration equations are: Peak Area = 64885.0 C_{ppm}+ 9285.8 for MET and Peak Area = 12414.6 C_{ppm}+ 11840.6 for MET and SIT, respectively. Based on the equations, the chromatographic sensitivity of MET is much better than SIT which may positively reflected on the quantitative analysis of this drug in real matrices. Detection and quantitation limits of both drugs are estimated based on the method of signal-to-noise ratio of 3 and 10, respectively. Table 2 shows that both drugs are quantified in their mixture down to 0.1 and 1.0 ppm for MET and SIT, respectively. SIT can be quantified in a wider range 2.0-150.0 mg/L. Ruggedness of the procedure is assessed by running different analysis over the same days and over three days as well.

The precision testes are carried out at solutions of 10.0 ppm (MET) and 100.0 ppm (SIT), respectively. The test includes six replicates. The proposed procedure shows high precision with overall RSD 6.6-8.3% for MET and 1.3-2.7% for SIT. The earlier data indicates the stability of solutions and the good repeatability of the proposed procedure. To assess the overall robustness of the proposed method, the main parameters are deliberately altered in the following manner: composition of mobile phase within ±10.0 % of acetonitrile, detection wavelength by ±3 nm, and pH of buffer by ±0.2. For detection wavelength, results indicates a convincing recovery and RSD for both drugs, 100.9 with RSD (0.2), and 99.9 with RSD (0.6) for MET and SIT, respectively. In the same meaning, changing the composition of organic part in the mobile phase do not highly affect the retention time and peak area of both drugs with final recovery of 101.9 with RSD (1.7), and 99.4 with RSD (0.2) for MET and SIT, respectively. It seems that changing pH of mobile phase by 0.2 unit do not affect the retention time, peak area, and resolution of both drugs with final results of 100.5 with RSD (0.4), and 99.6 with RSD (0.1) for MET and SIT, respectively. Overall, the method is stable and robust upon changing separation parameters which will support its application in real pharmaceutical analysis. The overall robustness of the proposed procedure produced RSD over the range 1.5-2.5%.

Quantification of MET and SIT in pharmaceutical matrices

Currently, the proposed analytical protocol will be used to separate and quantify both drugs in a prepared drugs formulation. Initially, the clean extract of the formulation is injected and a typical chromatogram is generated. This indicates the specificity of the method for both drugs. Moreover, the excipients do not affect the separation, retention times, shapes, and width of the eluted drugs. Figure 3 shows the separation of both drugs from the extract of commercial formulation. The accuracy/recovery and precision for MET and SIT

quantification in the formulation are provided in Table 3.

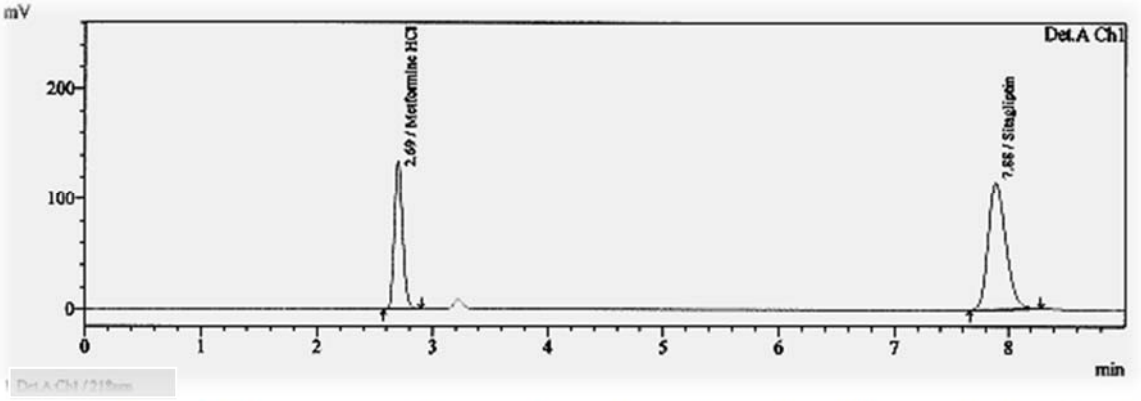


Figure 3: Measured chromatogram of drugs in the real formulation after dilution (detection 218 nm)

Figure 3 fairly indicates the efficiency of proposed chromatographic procedure for separation and detection of MET and SIT even in the presence of excipients that often added in the formulation including cellulose, polyvinylpyrrolidone, sodium lauryl sulfate, and sodium stearyl fumarate. The absence of any chromatographic signal for other constituents indicates the high selectivity for drugs, efficient matrix-cleaning procedure, and the poor detection of excipients at 218 nm. The noise-free and stable base line of the recorded chromatogram indicates absence of the other constituents in the final extracts. Moreover, the intense dilution of the extract (maximum dilution factor 50) will help to improve the signal of drugs against other excipients. As shown in Table 3, the proposed analytical procedure is workable for quantitative determination of both drugs in the real formulation which contains 50 mg MET and 500 mg SIT per tablet.

Table 3. Recovery of both drugs in real formulation using the proposed chromatographic technique

Metformin HCl			Sitagliptin		
Claimed level (mg)	**Found (mg)***	**Recovery %**	**Claimed level (mg)**	**Found (mg)***	**Recovery %**
50	49.8	99.6	500	498.0	99.6
50	50.2	100.3	500	498.0	99.6
50	50.6	101.1	500	505.0	101.0

* For drugs quantification in real formulation $n = 3$ and final RSD was less than 5%. The test was repeated three different times using fresh tablets.

It is worth to mention that the level of SIT was 10.0 times more than MET in the real formulation. Accordingly, the final extract is diluted 50 times to ensure that the final level of both drugs is within their dynamic ranges. The predicted level for both drugs is very close to the claimed value and this proves the accuracy of the proposed method. Moreover, the method was accurate and precise for assaying the commercial formulation which contains variable amounts of drug in one single run. In other common form which contains 50 MET and 1000 SIT per tablet, then different dilution should be applied and two chromatographic injections are necessary. For both drugs and at the best experimental conditions, the average recoveries were 100.3 and 100.1% for MET and SIT, respectively.

Stability-indicating capability and analytical selectivity of the proposed method for both drugs

Forced degradation is performed by exposing both drugs in real formulation to extreme or harsh experimental conditions such as heat, basic, acidic and oxidative environment. To assess thermal stability, the drugs are heated at 70 °C for three weeks. Effect of hydrolysis is investigated by contacting drugs with 1.0 N HCl and 1.0 N NaOH for 15 hours and at 70.0°C. Stability to light is investigated by irradiating solutions of drugs with UV at 765 W/m^2 for three weeks. Finally, oxidation by 1.0% H_2O_2 for 15 hours is also evaluated. Both peak area, retention time and detection new peaks are monitored in the stability studies. The extent of degradation of drugs s estimated from the peak intensities before and after applying the conditions. The overall results are provided in Table 4.

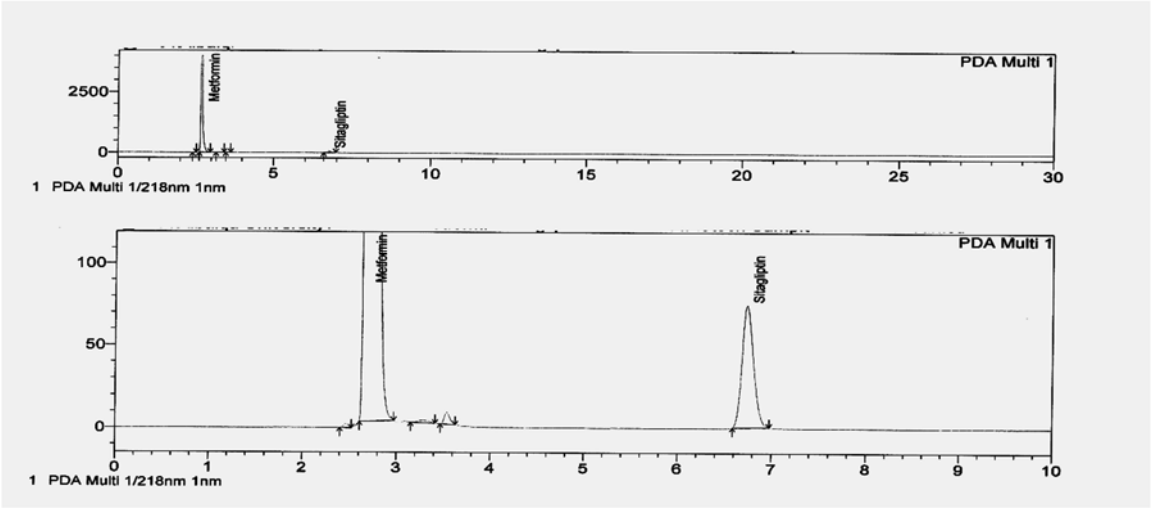
Table 4. Percentages of degradation of both drugs under different conditions. MET 10.0 µg/mL and SIT 100.0 µg/mL

Condition	Degradation%	
	MET	SIT
Thermal	3.4%	13.1%
Photolysis	31.1%	12.9%
Acidic	2.5%	25.3%
Basic	5.7%	19.7%
Oxidation	33.8%	17.5%

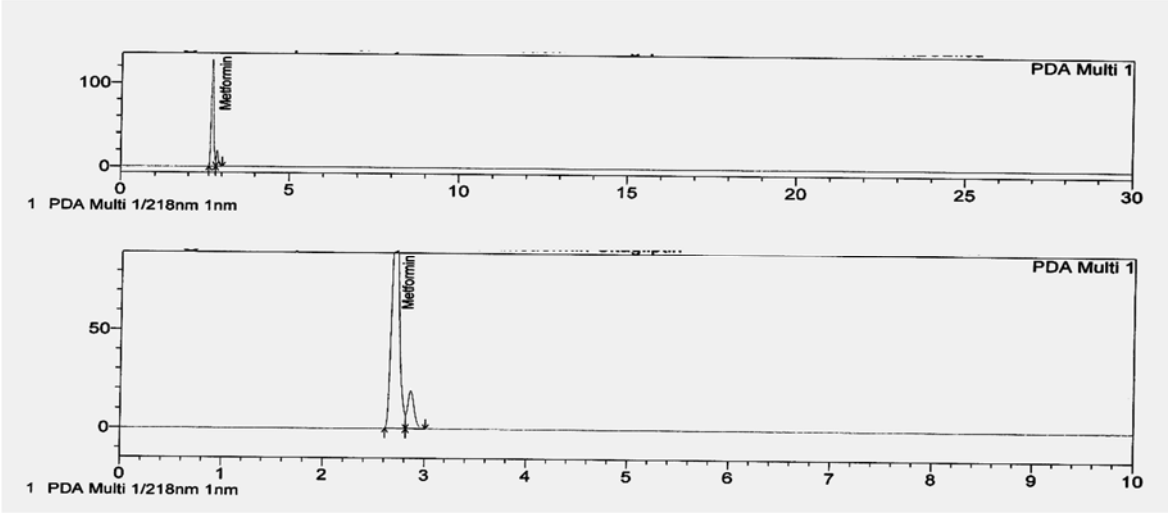
Table 4 shows that the degradation of MET at all conditions is acceptable and within range 2.5 (acidic environment Figure 4(a)) up to 33.8 (oxidation by H_2O_2 Figure 4(b)). Except for photolysis and oxidation, MET is more stable to other conditions as indicated from degradation% values. The drug MET is more stable than SIT in acidic conditions, Figure 4(a). The basic nature of SIT enhances the attack by acid which ended up with more degradation. SIT is more affected with solution acidity with degradation% values of 19.7 and 25.3 at basic and acidic conditions, respectively. Comparatively, both drugs are stable at high temperatures with degradation% values of 3.4 and 13.1 for MET and SIT, respectively. The maximum degradation is about 34% and observed when H_2O_2 is used to oxidize MET, Figure 4(b). The excess number of nitrogen (N) atoms in MET enhances the oxidation of the compound.

In terms of selectivity, the chromatograms of the samples are checked for the appearance of any extra peaks, injection of placebo, standard, active ingredients (separately) at concentrations of 10.0 and 100.0 µg/mL MET and SIT, respectively.

No chromatographic interference from any of the excipients is found at the retention times of the examined actives. In addition, the chromatogram of each active ingredient in sample and standard is found identical to the chromatogram of active drugs in single-injected runs. The sample and standard in addition to separate active ingredients are forced to vigorous degradations and injected in the chromatograph, no interference is observed from any degradant to the peaks of actives, the purity of MET and SIT peaks underwrote the selectivity of method.



(a)



(b)

Figure 4: (a) A typical representative chromatogram of Metformin HCl (1000 µg/ mL) and Sitagliptin (100 µg/ mL) treated with 1.0 N HCl. (b) A typical representative chromatogram of Metformin HCl (10 µg/ mL) treated with 1.0 % H_2O_2

4. Conclusion

Simultaneous quantification of MET and SIT drugs in commercial formulation is accomplished with minimum experimental efforts. The figures of merit confirm the simplicity (run time 9 min), precision (RSD < 5%), accuracy (recovery 100.2%), and reliable for quantification of drugs in real formulation. The proposed method is robust enough to be employed as stability-indicating for the drugs under different conditions. The results shows the acceptable stability of MET for acidic, basic, and thermal conditions. However, MET is unstable when exposed to UV and H_2O_2 with degradation% more than 30%. The results confirm that SIT was rather unstable at acidic, basic, and oxidation environments with degradation% of 17-25%. The proposed procedure can be extended to other combinations of the active ingredients in other doses.

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التحديد المتزامن للميتفورمين والسيتاجلبتين في الشكل الصيدلاني باستخدام الكروماتوغرافيا السائلة عالية الأداء

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ملخص

يستخدم هذا الدواء في علاج النوع الثاني من مرض السكري. قمنا في هذه الدراسة بتطوير طريقة تحليل بسيطة، دقيقة، صحيحة وذات فصل عالٍ باستخدام تقنية الفصل الكروماتوغرافي السائل عالي الأداء لفصل وتحديد كمية المواد الفاعلة لهذا الدواء في الشكل الصيدلاني.كما وقمنا باختبار ثباتية هذه الطريقة طبقاً لقواعد ICH.

في هذه الدراسة كان الطور المتحرك عبارة عن (العازل: أسيتونيتريل)–(70: 30) تم تعديل القوة الحمضية له لتصبح(6,0) وسرعة التدفق تساوي 1 مل/ دقيقة على طول موجة 218 نانوميتر. الفصل تم باستخدام عمود C8 طول 25 سم .

وجد بعد إجراء دراسات تحليلية وإحصائية للنتائج أن الطريقة المقترحة ذات علاقة خطية بالنسبة للمستحضرات خلال الفترة (1 – 15) ميكروغرام/ مل للميتفورمين وخلال الفترة (10 – 150) ميكروغرام/ مل للسيتاجليبتين. كما تبن أن صحة ودقة الطريقة كانت (1 – 15) ميكروغرام/ مل للمتفورمين، (2 – 150) ميكروغرام/ مل للسيتاجليبتين وأن حدود القياس والكمي لكلا ا لمستحضرين هي 1 ميكروغرام/ مل. ووجد أن المحاليل مستقرة لمدة ستة أيام، وبدراسة ثباتية المواد وجد أن الطريقة ذات انتقائية عالية لكلا المستحضرين.

في النهاية تم استخدام هذه الطريقه لفحص استقرار الشكل الصيدلاني تحت ظروف قاسية مصطنعة. بينت النتائج أن المتفورمين كان غير مستقر عند التعرض للأشعة فوق البنفسجية والH_2O_2.وكانت نسبة التفكك أكثر من 30%. كما وتبين أن السيتاجليبتين كان غير مستقر عند التعرض للوسط الحامضي أو القاعدي، أو تفاعلات التأكسد.

الكلمات الدالة: الميتفورمين، السيتاجليبتين، الكروماتوغرافيا السائلة عالية الأداء.

تاريخ استلام البحث 2017/1/12 وتاريخ قبوله للنشر 2017/2/13.