Development and Validation of New RP-HPLC Method for the Estimation of Antidiabetic Drugs Metformin Hydrochloride and Gemigliptin in Combined Pharmaceutical Dosage Form

Suleman S. Khoja* and Laxman J. Patel

1Faculty of Pharmacy, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Mehsana, Kherva-382711, Gujarat, India.

2Faculty of Pharmacy, Ganpat University, Mehsana, Kherva-382711, Gujarat, India.

Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i30B31649
(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.
(2) Smita Nayak, Gahlot Institute of Pharmacy, University of Mumbai, India.
(2) Alankar Shrivastava, KIET School of Pharmacy, KIET Group of Institutions, India.
Complete Peer review History: http://www.sciarticle4.com/review-history/69307

Received 25 March 2021
Accepted 02 June 2021
Published 05 June 2021

ABSTRACT

Metformin Hydrochloride and Gemigliptin is combination of Antidiabetic drug in tablet Zemimet SR © Tablet (25/500 mg), a member Antidiabetic drug, is a recent drug developed by LG Life sciences for the treatment of Type 2 diabetes. A new sensitive and rapid HPLC method was developed for the determination of Metformin Hydrochloride and Gemigliptin in pharmaceutical dosage forms; it was validated according to International Conference on Harmonization and Food and Drug Administration guidelines. The analysis was performed on the HPLC system equipped with a using Gemini C18, (5 μm) (250 mm x 4.6 mm), with of Buffer (20mM Ammonium Acetate in water, pH 3.5) and Methanol: Acetonitrile 40:10 (%V/V) 60: 40 v/v with at a flow rate of 1.0 mL/min, column temperature 35°C, total run time was 10 min, injection volume 10 μl, and detection was performed at the wavelength (λ) of 265 nm. The calibration plot gave linear relationship over the concentration range of Metformin Hydrochloride 20, 40, 100, 200, 400 and 500 μg/ml, and Gemigliptin 1, 2, 5, 10, 20 and 25 μg/ml, respectively. The accuracy of the proposed method was determined by recovery.

*Corresponding author: E-mail: premukhoja@gmail.com;
1.1.2 Gemigliptin

Gemigliptin is chemically 3(s)-3-amino-4- (5,5-difluoro oxopiperidino) [2,4 (trifluromethyl) -5,6,7,8 -tetra hydro [3,4-d] pyrimidine-7-yl] butan-1-one exhibits a unique structure. Gemigliptin (Zemiglo®, previously known as LC15-0444) has a different chemical structure compared to other DPP-4 inhibitors due to the presence of pyrimidine piperidine derivative as evident by X-ray crystallography. Gemigliptin binds to the S1, S2, and S2 extensive subsites of the DPP-4 enzyme. The piperidinone group of gemigliptin binds to the S1 subsite, where the upside F atom on the piperidin ring forms a hydrogen bond with the side chain of Tyr631 and the downside F atom makes a hydrophobic interaction with the side chain of Tyr666 and Tyr662. In addition, the key interaction occurs between the CF3 groups on the pyrimidinopiperidine and the S2 extensive subsite of the DPP-4 substrate, which enhances the potency of the drug and increases its selectivity as well. For Gemigliptin Molecular formula: \( C_{18}H_{19}F_{8}N_{2}O_{2} \), Molecular Weight: 489.36 g/mol. Gemigliptin L-tartrate Sesquihydrate Molecular formula: \( C_{18}H_{19}F_{8}N_{2}O_{2}C_{4}H_{6}O_{6} \cdot 1.5H_{2}O \), Molecular Weight: 666.4 g/mol.

![Fig. 2. Gemigliptin](image)

Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, “the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.” It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [4,8-13].

Literature survey revealed a few methods reported which are costly for routine testing for determination for Gemigliptin and Metformin in Pharmaceutical preparation [13-38].

In this research, a new sensitive and rapid HPLC method was developed for the determination of Gemigliptin and Metformin HCl in pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines.

---

**Keywords:** Metformin; gemigliptin; method development and validation; HPLC.

### INTRODUCTION

#### 1.1 Drug Profile

**1.1.1 Metformin Hydrochloride**

Metformin, chemically 1-carbamimidamido-N, N-dimethyl methanimidamide (Fig. 1) is a biguanide antihyperglycemic agent used for treating non-insulin dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Molecular formula: \( C_{6}H_{11}N_{5} \cdot HCl \), Molecular Weight: 165.63 g/mol [1-3].

![Fig. 1. Metformin Hydrochloride](image)

**1.1.2 Gemigliptin**

Gemigliptin is chemically 3(s)-3-amino-4- (5,5-difluoro oxopiperidino) [2,4 (trifluromethyl) -5,6,7,8 -tetra hydro [3,4-d] pyrimidine-7-yl] butan-1-one exhibits a unique structure. Gemigliptin (Zemiglo®, previously known as LC15-0444) has a different chemical structure compared to other DPP-4 inhibitors due to the presence of pyrimidine piperidine derivative as evident by X-ray crystallography. Gemigliptin binds to the S1, S2, and S2 extensive subsites of the DPP-4 enzyme. The piperidinone group of gemigliptin binds to the S1 subsite, where the upside F atom on the piperidin ring forms a hydrogen bond with the side chain of Tyr631 and the downside F atom makes a hydrophobic interaction with the side chain of Tyr666 and Tyr662. In addition, the key interaction occurs between the CF3 groups on the pyrimidinopiperidine and the S2 extensive subsite of the DPP-4 substrate, which enhances the potency of the drug and increases its selectivity as well. For Gemigliptin Molecular formula: \( C_{18}H_{19}F_{8}N_{2}O_{2} \), Molecular Weight: 489.36 g/mol. Gemigliptin L-tartrate Sesquihydrate Molecular formula: \( C_{18}H_{19}F_{8}N_{2}O_{2}C_{4}H_{6}O_{6} \cdot 1.5H_{2}O \), Molecular Weight: 666.4 g/mol.

![Fig. 2. Gemigliptin](image)
2. MATERIALS AND METHODS

2.1 Instrumentation
Chromatographic HPLC system equipped with a Gemini C18, (5 µm) (250 mm x 4.6 mm) column.

2.2 Chemicals and Reagents
Acetonitrile, Ammonium Acetate, Methanol, Water were of HPLC Grade.

2.3 Chromatographic Conditions
Mobile Phase (Buffer 20mM Ammonium Acetate in water, pH 3.5 and Methanol : Acetonitrile 40:10 (%V/V) in the ratio 60: 40 v/v with a flow rate of 1.0 mL/min, the detection was performed at the wavelength (\(\lambda\)) of 265 nm, injection volume 10 μl, run time 10 min, and column temperature 35°C Diluent –HPLC Grade water.

2.4 Preparation of Standard Solution
Weigh accurately and transfer about 25 mg of Gemigliptinand 500 mg of Metformin Hydrochloride standard into 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve, cool. Dilute to volume with diluent and mix. Transfer 4 ml of this solution to a 50 ml volumetric flask and dilute with diluent to volume and mix well.

2.5 Preparation of Sample Solution
Weigh accurately and transfer Approx. 595 mg of synthetic mixture (Equivalent to 25 mg of Gemigliptin and 500 mg of Metformin Hydrochloride) into 100 ml volumetric flask, add 50 ml of diluent and sonicate for 15 min with intermittent shaking. Dilute to volume with diluent and mix. Filter a portion of this solution using 0.45 µ PVDF Syringe filter, transfer 4 ml of this solution to a 50 ml volumetric flask and dilute with diluent to volume and mix well.

2.6 Method Validation
The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision, sensitivity (LOQ and LOD) robustness, and solution stability [5-7].

2.6.1 Specificity
Specificity is one of the significant features of HPLC, and it refers to the ability of the analytical method to discriminate between the analyte and the other components in the complex mixture [7]. Specificity of the method was evaluated by injecting 10 μL solutions of standard, sample, blank, and placebo separately.

2.6.2 Linearity
To evaluate the linearity and range of the method, Direct standard solutions were prepared by diluting the standard stock solution with the diluent in different concentrations Metformin Hydrochloride: 20, 40, 100, 200, 400 and 500 μg/ml, and Gemigliptin1, 2, 5, 10, 20 and 25 μg/ml which cover 70%, 90%, 100%, 110% and 120%, of the target concentration, respectively. Three replicate injections from each concentration were analysed under the same conditions. Linear regression analysis was used to evaluate the linearity of the calibration curve by using the least square linear regression method.

2.6.3 Sensitivity
Limit of detection (LOD)/limit of quantitation (LOQ) of Metformin Hydrochloride and Gemigliptin were determined by measuring the signal-to-noise ratio. limit of detection (LOD) is the concentration that gives a signal-to-noise ratio of approximately 3:1, while the limit of quantification (LOQ) is the concentration that gives a signal-to-noise ratio of approximately 10:1 with %RSD (n = 3) of less than 10%.

2.6.4 Accuracy
The accuracy of the assay method was determined by recovery studies at three concentration levels (80%, 100%, and 120%), i.e., 320, 400, and 480 μg/ml for Metformin Hydrochloride and Gemigliptin16, 20 and 24 μg/ml and three samples from each concentration were injected. percentage recovery of Metformin Hydrochloride and Gemigliptin added and RSD were calculated for each of the three replicate samples.

2.6.5 Precision
The system precision and method precision (repeatability) of the proposed methods were determined by several measurements of standard solution and sample solution, respectively [7-9]. System precision was established by six measurements of the standard
solution at the 100% concentration levels on the same day. Method precision was established by six assay determinations of the sample solution at the 100% concentration levels on the same day [9-12]. The RSD of obtained results was calculated to evaluate repeatability results.

2.6.6 Robustness

Robustness of the method was verified by applying minor and deliberate changes in the experimental parameters, for example:

(i) Column temperature: ±3°C
(ii) Flow rate: ±0.2 mL/min.
(iii) Change in pH.: ± 1

Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating % RSD and percent of recovery.

2.6.7 Stability of Analytical Solutions

The stability of analytical solutions was determined by analysing the standard and sample preparations at Initial, 12 Hr and 24 Hr at ambient room temperature 25°C. Three injections from each solution were analysed, and the average of the peak and the RSD were calculated.

3. RESULTS AND DISCUSSION

3.1 Method Development and Optimization

Several physical and chemical properties of Metformin Hydrochloride and Gemigliptin were obtained from the literature. The analytical method was developed to select preliminary reversed phase HPLC method chromatographic conditions, including detection wavelength, mobile phase, stationary phase, and sample preparation procedure. For this purpose, a series of trials were performed by varying the ratio of include trials.

Optimizing the chromatographic conditions on the Gemini C18 (5 µm), (250 mm x 4.6 mm column. the results of method optimization are summarized in Table 1. the mobile phase consisting of (Buffer 20mM Ammonium Acetate in water, pH 3.5 and Methanol : Acetonitrile 40:10 (%V/V) in the ratio 60: 40 v/v with a flow rate of 1.0 mL/min, injection volume 10 μl, run time 10 min, and column temperature 35°C at wavelength (λ) 265 was optimized as the best chromatographic conditions for the entire study where Metformin Hydrochloride and Gemigliptin was eluted forming symmetrical peak shape, resolution and suitable analysis time with retention time about 2.9 min for Metformin Hydrochloride (Met) and 7.4 min for Gemigliptin (Fig. 3) and Table 2.

| Column Used | Mobile Phase | Flow Rate | Wavelength | Observation |
|-------------|--------------|-----------|-------------|-------------|
| Gemini C18 (5 µm) (250 mm x 4.6 mm) | Water : Methanol (60:40) | 1.0 ml/min | 265 nm | Improper Peak Shape observed for both drugs |
| Gemini C18 (5 µm) (250 mm x 4.6 mm) | MeOH : Acetonitrile : 20 mM Ammonium Formate, pH 3.5 15:15: 70 v/v | 0.6 ml/min | 265 nm | Improvement was needed |
| Gemini C18 (5 µm) (250 mm x 4.6 mm) | MeOH : Acetonitrile : 20 mM Ammonium Formate, pH 3.5 25:15: 60 v/v | 0.6 ml/min | 265 | Improvement was needed |

Table 2. Result of optimized method

| Sr. No | Name          | Area   | Retention Time | Resolution | Tailing Factor | Theoretical Plate | Peak Purity Index |
|--------|---------------|--------|----------------|------------|----------------|-------------------|------------------|
| 1      | Metformin HCl | 36996  | 2.9 Min        | NA         | 1.3            | 6377          | 0.999995         |
| 2      | Gemigliptin   | 16009  | 7.4 Min        | 20.9       | 1.1            | 11379         | 0.999915         |
3.2 Method Validation

3.2.1 System Suitability

The % RSD for each parameter was found to be less than 2 %. This indicates the suitability of the system.

3.2.2 Specificity

Specificity was evaluated by comparing the chromatograms of mobile phase blank, placebo solution, standard solution, and sample solution (Metformin Hydrochloride and Gemigliptin). For this purpose, 10 μl from solutions mobile phase blank, standard solution (API) and sample solution were injected into the HPLC system separately, and the chromatogram results are shown in Figs. 4–5a, 5b, 5c, and 5d. It can be observed that there no coeluting peaks at the retention time of Metformin Hydrochloride and Gemigliptin interference. This result indicates that the peak of the analyte was pure and this confirmed the Specificity of the method.

Fig. 3. Chromatogram of Metformin Hydrochloride and Gemigliptin standard solution

Fig. 4. Chromatogram of Blank solution
Fig. 5a. Chromatogram of placebo solution

Fig. 5b. Chromatogram of Metformin API solution

Fig. 5c. Chromatogram of Gemigliptin API solution
3.2.3 Linearity and Range

Analytical method linearity is defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The mean peak area obtained from the HPLC was plotted against corresponding concentrations to obtain the calibration graph. The results of linearity study (Figs. 6 and 7) gave linear relationship over the concentration range of Metformin Hydrochloride: 20, 40, 100, 200, 400 and 500 μg/ml and Gemigliptin 1, 2, 5, 10, 20 and 25 μg/ml. From the regression analysis, a linear equation was obtained and the goodness-of-fit (r²) was found to be 0.99, indicating a linear relationship between the concentration of analyte and area under the peak.

3.2.4 Accuracy

The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy showed percentage recovery at all three levels in the range of for Metformin.
Hydrochloride 99.0 % to 101.0 % and Gemigliptin 98.0 % to 100.0%, and % RSD values were in the range of 0.70 - 0.93 % as shown in Table 3 and 4. The results of percentage recovery and %RSD were within the accepted limits from 98.0% to 102.0% and not more than 2.0%, respectively, which indicates the applicability of the method for routine drug analysis.

3.2.5 Precision

The precision of the method is deemed as “the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions,” and it is normally expressed as the relative standard deviation. The results of both system and method precision showed that the method is precise within the acceptable limits. The RSD, tailing factor, and number of theoretical plats were calculated for both solutions; all the results are within limits. Acceptable precision was not more than 2.0% for the RSD as shown in Tables 5 and Table 6.

![Graph of Gemigliptin calibration curve](image.png)

**Fig. 7. Standard calibration curve of Gemigliptin**

| % Level | Peak Area | Mean Area | Amount found | Amount added | % recovery |
|---------|-----------|-----------|--------------|--------------|------------|
| 80      | 95532     | 96029     | 321.01       | 320.200      | 100        |
|         | 96354     |           | 323.78       | 320.288      | 101        |
|         | 96201     |           | 323.26       | 320.448      | 101        |
| 100     | 119416    | 119316    | 401.27       | 400.176      | 100        |
|         | 118463    |           | 398.07       | 400.704      | 99         |
|         | 120071    |           | 403.47       | 400.184      | 101        |
| 120     | 143299    | 143796    | 481.52       | 480.264      | 100        |
|         | 144731    |           | 486.34       | 480.184      | 101        |
|         | 143360    |           | 481.73       | 480.704      | 100        |
| Acceptance: -98.0 to 102.0 | | | | | |
| % RSD: - NMT 2.0 % | | | | | |

**Table 3. Metformin Hydrochloride Recovery data of the proposed HPLC method**

- **y = 1594x - 103.98**
- **R² = 0.9998**
Table 4. Gemigliptin Recovery data of the proposed HPLC method

| % Level | Peak Area | Mean Area | Amount found | Amount added | % recovery |
|---------|-----------|-----------|--------------|--------------|------------|
| 80      | 26511     | 26254     | 16.05        | 16.088       | 100        |
|         | 26012     |           | 15.75        | 16.064       | 98         |
|         | 26239     |           | 15.89        | 16.176       | 98         |
| 100     | 32479     | 32733     | 19.67        | 20.040       | 98         |
|         | 32583     |           | 19.73        | 20.192       | 98         |
|         | 33139     |           | 20.07        | 20.048       | 100        |
| 120     | 39766     | 39582     | 24.08        | 24.040       | 100        |
|         | 39403     |           | 23.86        | 24.096       | 99         |
|         | 39579     |           | 23.97        | 24.128       | 99         |

Acceptance: - 98.0 to 102.0
% RSD: - NMT 2.0 %

Table 5. System Precision data from standard solution of the proposed HPLC method

| Replicate No. | Metformin hydrochloride area | Gemigliptin Area |
|---------------|------------------------------|------------------|
| 1             | 63297                        | 16605            |
| 2             | 62576                        | 16341            |
| 3             | 63124                        | 16587            |
| 4             | 62846                        | 16400            |
| 5             | 62868                        | 16345            |
| 6             | 62755                        | 16481            |
| Mean          | 62911                        | 16460            |
| SD            | 260                          | 117              |
| % RSD         | 0.40                         | 0.70             |

3.2.6 Robustness

The analytical method robustness was tested by evaluating the influence of minor modifications in HPLC conditions on system suitability parameters of the proposed method, as mentioned in Section 2.6.6. The results of robustness testing showed that a minor change of method conditions, such as the variation of the temperature and flow rate, is robust within the acceptable limits. The results are summarized in Table 7. In all modifications, good separation of Metformin Hydrochloride and Gemigliptin was achieved, and it was observed that the percent of recovery was within acceptable limits and the %RSD is within limit of not more than 2.0 %. Acceptable limits as well. The results are shown in Table 7.

Table 6. Method Precision data from Sample solution of the proposed HPLC method

| Replicate No. | Metformin hydrochloride area | Gemigliptin area |
|---------------|------------------------------|------------------|
| 1             | 63300                        | 16605            |
| 2             | 62569                        | 16441            |
| 3             | 63102                        | 16587            |
| 4             | 62896                        | 16469            |
| 5             | 62895                        | 16496            |
| 6             | 62754                        | 16489            |
| Mean          | 62919                        | 16514            |
| SD            | 256.70                       | 66.19            |
| % RSD         | 0.40                         | 0.40             |

3.2.7 Solution stability

The percent of recovery was within the range of 98.0% to 102.0% and RSD was not more than 2.0%, indicating a good stability of the sample and standard Solutions for 0 Hr, 12 Hr and 24 Hr at Room Temperature (RT) conditions. The peak area was as comparable to standard and percent of recovery was within acceptable limits, and the % RSD is within the limit of not more than 2.0%. The results are shown in Table 8.
Table 7. Robustness data of the proposed HPLC method

| Parameter     | Condition | Peak Area | % RSD |
|---------------|-----------|-----------|-------|
|               |           | Metformin HCl Area | Gemigliptin Area | Metformin HCl | Gemigliptin |
| Column        | 32 °C     | 61952     | 16552  | 0.80 | 1.59 |
| Temperature   | 35 °C     | 62219     | 16489  | 0.40 | 0.40 |
| ±3 °C         | 38 °C     | 63409     | 16675  | 0.56 | 0.90 |
| Flow Rate     | 0.9 ml/min| 76058     | 19826  | 0.80 | 1.16 |
| ±0.1 ml/min   | 1.0 ml/min| 62919     | 16489  | 0.40 | 0.40 |
|               | 1.1 ml/min| 62040     | 16535  | 0.37 | 1.34 |
| Change in pH  | 3.4pH     | 69643     | 18290  | 0.51 | 1.20 |
|               | 3.5pH     | 62919     | 16489  | 0.40 | 0.40 |
| ± 0.1 pH      | 3.6pH     | 70591     | 18415  | 0.37 | 0.53 |

Table 8. Solution stability data of the proposed HPLC method

| Parameter       | Time Point | Peak Area | % RSD |
|-----------------|------------|-----------|-------|
|                 |            | Metformin Hydrochloride Area | Gemigliptin Area |
| Standard Solution | 0 Hr (Initial) at RT | 62911 | 16460 |
|                 | After 12 Hr at RT | 62093 | 16357 |
|                 | After 24 Hr at RT | 62061 | 16332 |
| Sample Solution | 0 Hr (Initial) at RT | 62919 | 16489 |
|                 | After 12 Hr at RT | 63637 | 16747 |
|                 | After 24 Hr at RT | 71268 | 18593 |

4. CONCLUSION

In the present research, a fast, simple, accurate, precise, and linear HPLC method has been developed and validated for quantitative analysis of Zemimet SR ® Tablet (25/500 mg) combined dose and formulation, and hence it can be employed for routine quality control analysis for finished and stability sample analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Metformin Hydrochloride and Gemigliptin. In addition, the main features of the developed method are short run time and retention time around 2.9 min for Metformin Hydrochloride (Met) and 7.4 min for Gemigliptin. The method was validated in accordance with ICH/FDA guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions.

DATA AVAILABILITY

Data available through correspondence if required.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

Authors are thankful to Department of Pharmaceutical Sciences, Saurashtra University, Rajkot and Special thanks to Dr. Mihir Raval and Dr. Trupesh Pethani providing facility to do project work and support in research also thankful to Ganpat University faculty for...
Authors have declared that no competing interests exist.

REFERENCES

1. Meher Vijay Dalawai et al. Development and validation of stability indicating assay method by HPLC for the analysis of sitagliptin phosphate in bulk drug substances, Journal of Chemical and Pharmaceutical Research. 2015;7(10):781-787.
2. Vasanth PM, et al. Method development and validation of sitagliptin and metformin using reverse phase HPLC method in bulk and tablet dosage form, Der Pharmacia Lettre. 2013; 5(5):168-174.
3. Hanan A. et al. May, Nesrin K. Ramadan, Sherine S. Diab, Azza A. Moustafa Chromatographic methods for the simultaneous determination of binary mixture of Saxagliptin HCl and Metformin HCl, Bulletin of Faculty of Pharmacy, Cairo University. 2017;55(2):311-317.
4. Konari SN. Stability indicating validated RP-HPLC technique for the analysis of multicomponent anti-diabetic drug combos in pharmaceutical dosage forms, Karbala. International Journal of Modern Science. 2015;1:39-48.
5. Deepti V. Stability-indicating RP-HPLC method for analysis of sitagliptin in the bulk drug and it’s pharmaceutical dosage form. Available:https://www.pharmatutor.org/articles/stability-indicating-rp-hplc-method-analysis-sitagliptin-bulk-drug-pharmaceutical-dosage-form
6. Lavanya R, Yunoos MD. Development and validation of RP-HPLC method for the estimation of sitagliptin phosphate in bulk and its tablet dosage form. J. Adv. Pharm. Edu. & Res. 2013; 3(4):475-479.
7. Mohamed Karam Qassas. A validated HPLC stability indicating method for the determination of sitagliptin in bulk drug substance and tablets. Int. J. Pharm. Sci. Rev. Res. 2015;32(1):33:194-19.
8. Hitesh P. Inamdar, RP-HPLC method for simultaneous determination of metformin hydrochloride, rosiglitazone and sitagliptin – application to commercially available drug products. IJPSR. 2012;3(9):3267-3276. Doi:http://dx.doi.org/10.13040/IJPSR.0975-8232.3 (9).3267-76.
9. Scheen AJ. DPP-4 inhibitors in the management of type 2 diabetes: a critical review head-to-head trials. Diabetes Metab. 2012;38:89-101.
10. Chen K, Kang D, Yu M, et al. Direct head-to-head comparison of glycaemic durability of dipeptidyl peptidase-4 inhibitors and sulphonylureas in patients with type 2 diabetes mellitus: A meta-analysis of long-term randomized controlled trials. Diabetes Obes Metab. 2017;published on line. DOI: 10.1111/dom.13147.
11. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2015;38:140-49.
12. Garber AJ, Abrahamson MJ, Barzilay JI, et al. Consensus statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the comprehensive type 2 diabetes management algorithm - 2017 executive summary. Endocr Pract, 2017;23:207-38.
13. American Diabetes Association. Standards of medical care in diabetes - 2017. Diabetes Care. 2017;40:S1-S135.
14. Qaseem A, Barry MJ, Humphrey LL, et al. Oral pharmacologic treatment of type 2 diabetes mellitus: a clinical practice guideline update from the American College of Physicians. Ann Intern Med. 2017;166:279-90.
15. Scott L. Sitagliptin: a review in type 2 diabetes. Drugs. 2017;77:209-24.
16. Luhar SV, Patel KR, Jani GK, Narkhede SB. Stability study of gemigliptin and simultaneous estimation of gemigliptin and its degradation product by RP-HPLC Method. JPharm SciBioscientific Res. 2016;6(3):338-346.
17. GampaVijaya Kumar, et al. Analytical method development and validation for the simultaneous estimation of metformin gemigliptin by RP-HPLC Method. Int. J. Med. Pharm. Res. 2016;4(6):321-330.
18. Scheen AJ. A review of gliptins in 2011. Expert Opin Pharmacother. 2012;13:81-99.
19. Scheen AJ. A review of gliptins for 2014. Exp Opin Pharmacother. 2015;16:43-62.
20. Shailesh V. Luhar, bioanalytical method development and validation of gemigliptin tartrate sesquihydrate. ejbps. 2017;4(4):488-501.
21. Krishna Y, Kedhareshwari R, Harikha Y, Harikrishna V, Muzahid K Gopi. Analytical method development and validation for the simultaneous estimation of metformin and gemigliptin by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form, Pharma research Library; 2017.
22. Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of Dipeptidyl peptidase-4 inhibitors. Endocr Rev. 2014;35:992-1019.
23. Venkateswara Rao P, et al. A new stability indicating Rp-Hplc method for simultaneous estimation of ertugliflozin and sitagliptin in bulk and pharmaceutical dosage form its validation as per ICH guidelines. Indo Am. J. P. Sci. 2018;05(04).
24. Shyamala M, Mohideen S, Satyanarayana T, Narasimha Raju Ch, Suresh Kumar P, Swetha K. Validated RP-HPLC for simultaneous estimation of sitagliptin phosphate and metformin hydrochloride in tablet dosage form. American Journal of Pharm Tech Research. 2011;1,2:93-101.
25. Ramzia El-Bagary I, Ehab Elkady F, Bassam Ayoub M. Spectrofluorometric and spectrophotometric methods for the determination of sitagliptin in binary mixture with metformin and ternary mixture with metformin and sitagliptin alkaline degradation product. International Journal of Biomedical Sciences. 2011;7(1):62-69.
26. Miller S, Krumins T, Zhou H, et al. Ertugliflozin and Sitagliptin co-initiation in patients with type 2 diabetes: the VERTIS SITA randomized study. Diabetes Ther. 2018;9(1):253-268.
27. US FDA. Guideline for industry: Bioanalytical Method Validation, 21-May 2018.
28. Snyder L. Practical HPLC Method Development; 2ndEdn; Wiley Inter Science Publication, USA, 1997; 723-754.
29. Snyder L. Practical HPLC Method Development; 2ndEdn; Wiley Inter Science Publication, USA, 1997;089-099.
30. ICH Q1A (R2) Stability Testing of new Drug Substances and Products.
31. Bakshi M, Sing S, Saranjit Singh. Development of validated stability-indicating assay methods-critical review. J. of Pharm. and Biomed. Anal. 2002;28:1011–1040.
32. ICH, Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Geneva; 2005.
33. United state pharmacopeia; 41 The United states Pharmacopoeialconvention. 7665-7670.
34. Development and Validation of an HPLC Method for Determination of Antidiabetic Drug Alogliptin Benzoate in Bulk and Tabletshttps://www.hindawi.com/journals/jamc/2018/1902510/
35. Available:https://www.researchgate.net/publication/321311777_Gemigliptin_Newer_Promising_Gliptin_for_Type_2_Diabetes_Mellitus/fulltext/5a4bc292a6fdcc3e99cf5c38/Gemigliptin-Newer-Promising-Gliptin-for-Type-2-Diabetes-Mellitus.pdf
36. Available:https://www.ijem.in/article.asp?is sn=2230-8210;year=2017;volume=21;issue=6;spage=898;epage=902;aulast=Gutch
37. A Simple and Validated Rp-Hplc Method for The Simultaneous Determination Of Vildagliptin And Metformin In Bulk And Pharmaceutical Dosage Forms769.pdf (innovareacademics.in)
38. Available:https://diabetestalk.net/diabetes/how-does-metformin-decrease-intestinal-absorption-of-glucose

© 2021 Khoja and Patel; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.