# REVIEW ON ANALYTICAL METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND SITAGLIPTIN IN BULK AND TABLET FORMULATION BY RP-HPLC

**Deepak M, Vijey Aanandhi M***
**Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India. Pin code- 600117**

**Corresponding Author: Dr.M.Vijey Aanandhi**
**Professor and Head,**
**Department of Pharmaceutical Chemistry and Analysis,**
**School of Pharmaceutical Sciences,**
**Vels Institute of Science, Technology and Advanced Studies (VISTAS),**
**Pallavaram.Chennai-600 117.**
**Mobile: 9840959519**
**Email: hodpchemistry@velsuniv.ac.in**



**ABSTRACT**
An overview about simultaneous estimation of the combination drugs, metformin and sitagliptin using RP-HPLC method. Reverse phase chromatography is the most commonly used separation technique in HPLC, common reasons being its simplicity, versatility and its ability to handle compounds of a diverse polarity and molecular mass.Good knowledge about different types of mobile phases and their combination are requiredfor highly precise and accurate method development. The retention time and linearity of metformin and sitagliptin are found to be determined underdifferent chromatographic conditions such as column, mobile phase, elution mode, flow rate and wavelength detected using UV detector. In this article, we will be reviewing different developed methods for estimating the given combination drugs by RP-HPLC.

**KEY WORDS:** Metformin hydrochloride, Sitagliptin Phosphate, HPLC, Chromatographic conditions, Retention Time, Linearity.
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## INTRODUCTION:

Metformin hydrochloride is an oral hypoglycemic agent that works by decreasing glucose production in the liver and decreasing absorption of glucose by the intestines. It is chemically known as*N*, *N*-dimethyl imido dicarbonimidic diamide hydrochloride, white to off-white crystalline, non-hygroscopic powder.It is freely soluble in water, slightly soluble in Alcohol and is practically insoluble in acetone,methylene chloride, ether, and chloroform (Sudhir Adsul et al., 2018). Molecular formula for the compound is C4H11N5.HCl and themolecular weight is

165.63g/mol.Metformin is prescribed as a first-line therapy in type-2 diabetes.It exerts the glucose-lowering effect (i) via inhibition of gluconeogenesis in the liver, (ii) by delaying the action of glucagon, (iii) by facilitating the action of insulin, and (iv) by delaying glucose absorption from the intestine (P. Santosh Kumar et al., 2022).Its structural formula is presented in Figure 1.

Sitagliptin phosphate is an orally active Dipeptidyl peptidase 4 (DPP-4) inhibitor, that works by regulating the levels of insulin your body procedure after eating. It also enhances glycemic control and shows a positive influence on the growth of *β*-cells in pancreatic islets.It is chemically 7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl) butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*] pyrazine phosphate (1:1)monohydrate. It is awhite to off-white crystalline, non-hygroscopic powder that is soluble in water and N, N-dimethyl formamide, slightly soluble in methanol and very slightly soluble in ethanol, acetone, and acetonitrile and insoluble in isopropanol (Nagunath Sirigiri et al., 2018). Empirical formula of the compound is C16H15F6N5O.H3PO4.H2O, molecular weight is 523.32 g/mol.Sitagliptin is used for the treatment of type 2 diabetes. It is effective in lowering HbA1c, fasting as well as postprandial glucose in monotherapy and in combination with other oral anti diabetic agents (P. Santosh Kumar et al., 2022).its structural formula is presented in Figure 2

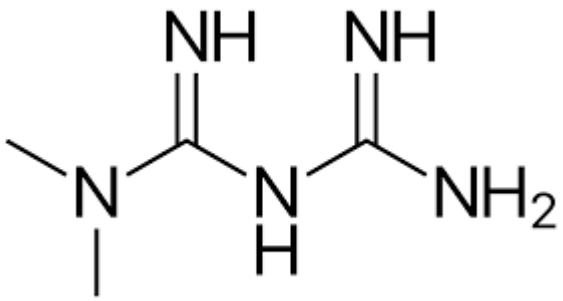


*Figure 1:* Metformin

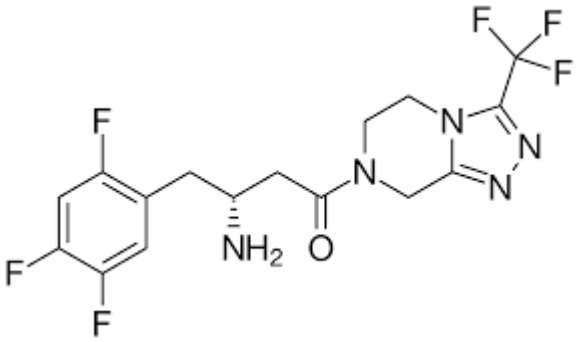


*Figure 2:* Sitagliptin



**DEVELOPED METHODS**

Different methods for simultaneous estimation of metformin and sitagliptin from literature review are as follows.

| METHOD | CHROMATOGRAPHIC CONDITION | OBSERVATION | REFERENCE |
|---|---|---|---|
| 1 | **Column** : Grace C18 column – 250nm X 4.6 ID, Particle size: 5 micron<br>**Mobile Phase** : Methanol : HPLC grade water (80:20% v/v, pH - 3.0)<br>**Elution Mode** :Isocratic<br>**Flow Rate** :0.8ml/min<br>**Detector** :UV detector at 254nm | The retention time for metformin and sitagliptin is 6.19 min and 7.42 min. The calibration curve was linear at the concentration range 5 – 50 µg/ml of both metformin and sitagliptin. The LOD and LOQ of metformin and sitagliptin was within the rage, the percentage recovery of metformin is 99.8% and sitagliptin is 100%. | Sudhir Adsul et al., 2018 |

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| 2 | **Column :** Zorbax SB-C8, 150 X 4.6 mm, 3.5 µm<br>**Mobile Phase :**Methanol : Buffer (40:60% v/v, pH - 5.0), Buffer - 1.54 g of ammonium acetate and 0.58 g Octane-1-sulfonic acid sodium salt, dissolve in 1000 mL of Milli-Q-Water and adjust the pH to 5.0 with glacial acetic acid.<br>**Elution Mode :** Isocratic<br>**Flow Rate :**1 ml/min<br>**Injection Volume :**10µl<br>**Temperature :** 35ºC<br>**Detector :**UV detector at 225nm for metformin & 265nm for sitagliptin | The retention time for metformin and sitagliptin are 2.398 min and 17.113 min. The method is linear in a range of 50% to 150% doe both drug. | Nagunath Sirigiri et al., 2018 |
| 3 | **Column :** Avantor, ACE C18 (Length 150 x Diameter 4.6mm Particle size 5µm)<br>**Mobile Phase :**<br>Buffer : Methanol (90:10), Buffer : 2.8g/L trisodium phosphate and 2g/L of 1-heptane sodium salt anhydrous (Adjust the pH 3.5 ± 0.2 with diluted phosphoric acid)<br>Acetonitrile : Methanol (30:70)<br>**Elution Mode :**Gradient flow<br>**Flow Rate :**1.2 ml/min<br>**Injection Volume :**10µl<br>**Temperature :** 35ºC<br>**Detector :**UV detector - 210nm | The retention time for metformin and sitagliptin are 2.112 min and 10.975 min. Linear calibration curves with good correlation coefficients were obtained over the concentration ranges of 50 – 200 µg/mL for both drugs. | P. Santosh Kumar et al., 2022 |
| 4 | **Column :**Alltima C18 (150 x 4.6 mm, 5µm)<br>**Mobile Phase :**Methanol : Buffer (20:80 v/v), Buffer: 2 mL of ortho phosphoric acid in 1000 mL of water; pH 2.4<br>**Elution Mode :**Isocratic<br>**Flow Rate :**1 ml/min<br>**Injection Volume :**10µl<br>**Temperature :**30ºC<br>**Detector :**UV detector - 210nm | The retention time for metformin and sitagliptin are 2.35 min and 3.04 min. The linearity of this method was found in the concentration range of 50 µg/mL to 300 µg/mL for Metformin and 5 µg/mL to 30 µg/mL Sitgliptin.The LOD of metformin is 0.38µg/ml and sitagliptin is 0.09µg/ml, The LOQ of metformin is 1.14µg/ml and sitagliptin is 0.58µg/ml. | Padmalatha H and Haris M 2015 |

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| 5 | **Column** : Hypersil BDS C18 (150 x 4.6 mm, 5 µ)<br>**Mobile Phase** : phosphate buffer: methanol (50:50), Buffer: 2.87g of potassium dihydrogen phosphate in 1000ml of HPLC grade water, pH 4 with orthophosphoric acid.<br>**Elution Mode** : Isocratic<br>**Flow Rate** :1 ml/min<br>**Injection Volume** : 20µl<br>**Temperature** :<br>**Detector** :UV detector - 260nm | The retention time was found to be 1.773 min for Metformin and 3.696 min for Sitagliptin, Linearity was observed over a concentration range of 50 to 150µg/ml for Metformin and 5 to 15 µg/ml for Sitagliptin. System suitability parameters are satisfactory and resolution was >2. The theoretical plates are > 2000. Tailing factor <2. %RSD not more than 2%. | Rani Sirisha G et al., 2013 |
| 6 | **Column** :Lichrosphere-100 C18 ODS (250 × 4.6 mm, 5 µm)<br>**Mobile Phase** : methanol and potassium di-hydrogen phosphate buffer (70:30 v/v)<br>**Elution Mode** : Isocratic<br>**Flow Rate** :1 ml/min<br>**Injection Volume** :20µl<br>**Temperature** :30ºC<br>**Detector** :UV detector - 261nm | The retention times of metformin hydrochloride and sitagliptin phosphate are 4.9 and 6.1 min. Linear calibration curves with good correlation coefficients were obtained over the concentration ranges of 10 – 50 µg/mL for sitagliptin and 20 - 100 µg/mL for metformin. The LOD of metformin is 0.14µg/ml and sitagliptin is 0.016µg/ml, The LOQ of metformin is 0.42µg/ml and sitagliptin is 0.048µg/ml. | P. B. N. Prasad et. al., 2014 |
| 7 | **Column** :Luna C18 (250mmx4.6mm, 5µ particle size)<br>**Mobile Phase** :Buffer : aceton (50:50), Buffer - Ortho Phospharic acid is dissolved in 1000 ml water.<br>**Elution Mode** : Isocratic<br>**Flow Rate** :1 ml/min<br>**Injection Volume** :20µl<br>**Detector** :UV detector - 285nm | The retention time for metformin and sitagliptin is 3.98 min and 5.75 min. Linear correlation was obtained between peak area Vs concentration of Metformin and Sitagliptin were in the range of 10.01-150.15µg/mL and 1.06-15.02 µg/mL. The LOD of metformin is 0.1485µg/ml and sitagliptin is 0.00716µg/ml, The LOQ of metformin is 1.0876µg/ml and sitagliptin is 0.0147µg/ml. | Srivani Mallepelli et. al., 2017 |

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| 8 | **Column** :Hypesil BDS C18 Column (100x 4.6 mm,5µm)<br>**Mobile Phase** :potassium dihydrogen orthophosphate : methanol(50:50v/v),adjusted the pH 8.5 with o-phosphoric acid<br>**Elution Mode** :Isocratic<br>**Flow Rate** :1 ml/min<br>**Injection Volume** :10µl<br>**Temperature** :30ºC<br>**Detector** :UV detector - 215nm | The retention time for metformin and sitagliptin is 4.6 min and 2.3 min. The calibration curve was linear at the concentration range 50 – 150 µg/ml of both metformin and sitagliptin. The LOD of metformin is 0.08µg/ml and sitagliptin is 0.07µg/ml, The LOQ of metformin is 2.6µg/ml and sitagliptin is 2.3µg/ml. | Karimulla S K et. al., 2013 |
| 9 | **Column** :C18 Monolithic column (100mm× 4.5mm i.e., 5µm) connected with an C18 guard cartridge (4mm×3mm i.d., 5µm).<br>**Mobile Phase** : methanol : acetonitrile: potassium dihydrogen orhtophosphate(42.135:10:47.865 %v/v, pH 3.5 )<br>**Elution Mode** :Isocratic<br>**Flow Rate** :0.3 – 0.5 ml/min<br>**Injection Volume** :20µl<br>**Temperature** :20ºC<br>**Detector** : UV detector - 210nm | The retention time for metformin and sitagliptin is 3.3 min and 4.4 min. | Balamurugan Krishnan, Kirtimaya Mishra 2020 |



## CONCLUSION

Analytical method development using RP-HPLC are allsaid to be highly accurate, specific, simple and cost effective. Fromthe above mentioned methods, method5 shows the least retention time of 1.773 min for metformin and 3.696 min for sitagliptin respectively, where 50% of Methanol and 50% of Potassium dihydrogen orthophosphate buffer were used as mobile phases for elution and peak was observed at 260nm in an UV detector. Following this, method 4 shows second least retention time of 2.35 min for metformin and 3.04 min for sitagliptin where 20% of Methanol and 80% of Ortho Phosphoric Acid buffer were used as mobile phases for elution and the peak was observed at 210nm in UV detector. Finally, method 9 showed a retention time of 3.3min for metformin and 4.4 min for sitagliptin where 42.135% of Methanol, 10% of Acetonitrile and 47.865% of Potassium dihydrogen orthophosphate buffer were used as mobile phase for elution and peak observed at 210nm in UV detector. From this review, we can get an overall idea of differentcombination of mobile phases that can be used for simultaneous estimation of Metformin and Sitagliptin.

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