# ANALYTICAL METHODS DEVELOPMENT AND VALIDATION OF COMBINATION OF TWO DRUGS BY RP HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

**Pankaj Sharma[1*], Prof. (Dr.) Rajesh Verma[2] , Prof. (Dr.) Pankaj Kumar Sharma[3], Dr. Jaya Sharma[4]**

**Abstract:**
This research paper focuses on the development and validation of a robust and efficient RP-HPLC method for the simultaneous estimation of drug combinations in marketed formulations. The study is divided into two parts, addressing the simultaneous estimation of Metformin & Sitagliptin, and Metformin & Saxagliptin, respectively. The analytical parameters evaluated for method validation include system suitability, specificity, linearity, accuracy, limit of quantification (LOQ), limit of detection (LOD).

**Keywords:** Drug, Sitagliptin, Metformin, Saxagliptin.

[1*]Research Scholar, Department of Pharmacy, Apex University, Jaipur
[2]Professor, Department of Pharmacy, Apex University, Jaipur
[3]Dean Pharmacy, Department of Pharmacy, Apex University, Jaipur
[4]Professor and Principal, Department of Pharmacy, Apex University, Jaipur

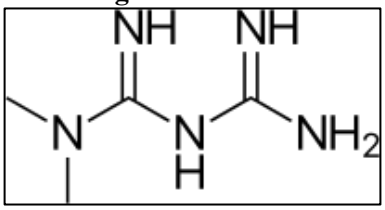
***Corresponding Author:** Pankaj Sharma
*****Research Scholar, Department of Pharmacy, Apex University, Jaipur

**DOI:** - 10.48047/ecb/2023.12.Si6.732

**Introduction:**

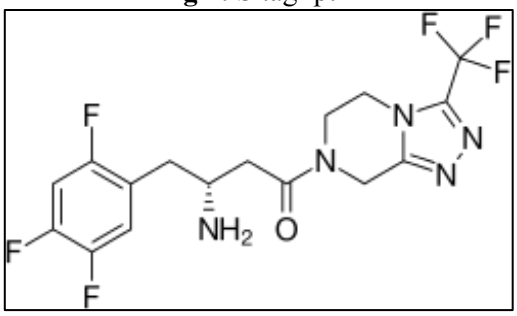
Metformin is a medication commonly used as a hypoglycemic agent in the treatment of diabetes. The chemical name of Metformin is N,N-Dimethylimidodicarbonimidic diamide, with a molecular formula of $C_4H_{11}N_5$ and a molecular weight of 129. The recommended dose of Metformin is 500mg. Metformin is primarily eliminated from the body through the kidneys (renal elimination). Like any medication, Metformin can have adverse effects or side effects. Discomfort in the abdomen or stomach, a sore throat, loss of appetite, diarrhoea, rapid or shallow breathing, a high body temperature, chills, fever, pain in the lower back or side, cramping or pain in the muscles, trouble urinating, and drowsiness are some of the more common side effects. Anxiety, blurred vision, chest pain, cold sweats, unconsciousness, disorientation, chilly and pale complexion, sadness, and difficulty or laboured breathing are among the less frequent adverse effects described.

**Fig 1:** Metformin




Sitagliptin is a medication used as a hypoglycemic agent in the treatment of diabetes. It has a molecular formula of $C_{16}H_{15}F_6N_5O$ and a molecular weight of 407.31. The chemical name of sitagliptin is (R) -4-oxo-4-[3- (trifluoromethyl) -5,6-dihydro [1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.
Sitagliptin exerts its therapeutic effects by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4). While taking sitagliptin, individuals may experience certain side effects. These can include nausea.

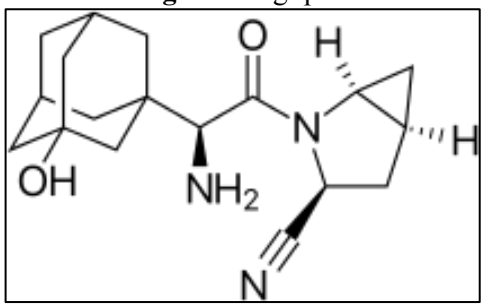
**Fig 2:** Sitagliptin




Saxagliptin is a medication used for the treatment of diabetes. It falls under the category of dipeptidyl-peptidase IV (DPP-4) inhibitors, which are commonly used drugs in diabetes management. The chemical name of saxagliptin is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-

carbonitrile. It has a molecular formula of $C_{18}H_{25}N_3O_2$ and a molecular weight of 315.41. Saxagliptin is typically prescribed in doses of either 2.5 mg or 5 mg. After oral administration, it is absorbed within approximately 2 hours. The elimination of saxagliptin occurs primarily through renal clearance. As with any medication, saxagliptin can have adverse effects or side effects. Colitis and diarrhea are among the reported adverse effects associated with saxagliptin use.

**Fig 3:** Saxagliptin




**Experimental:**

The present study was divided into two parts and designed for the simultaneous estimation of drug combinations in marketed formulations. The first part deals with development and validation of HPLC method for the simultaneous estimation of Metformin & Sitagliptin in marketed formulations. The second part deals with Metformin & Sitagliptin in the same context.

**Research Methodology:**

**1. Preparation of Solutions for Metformin & Sitagliptin:**

**a. Buffer solution (0.01N $KH_2PO_4$ in case of Metformin & Sitagliptin and 0.02N $KH_2PO_4$ in case of Metformin and Saxagliptin):** For Metformin & Sitagliptin, accurately weighed 1.36gm of sodium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 3.3 with dil. OPA.

For Metformin and Saxagliptin, accurately weighed 2.72gm of sodium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 4.8 with dil. OPA.

**b. Standard Stock Solution:** For Metformin & Sitagliptin, accurately Weighed and transferred 500mg of Metformin, 50mg of sitagliptin working Standards into a 100ml clean dry volumetric flask respectively, add diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluents.

For Metformin and Saxagliptin, accurately Weighed and transferred 500mg of Metformin, 5mg of saxagliptin working Standards into a 100ml clean dry volumetric flask Respectively, add diluent, sonicated for 30 minutes and make up to the final volume with diluents.

**c. Working Standard Solutions:** Aliquots of 0.25, 0.5, 0.75, 1.0, 1.25 & 1.5 ml were pipetted out from the stock solution and transferred into a 10 ml volumetric flask and volume was made up to 10 ml with diluent. This gives the solutions of 12.5, 25, 37.5, 50, 62.5, 75 µg/ml for Metformin and 1.25, 2.5, 3.75, 5.0, 6.25, 7.5µg/ml for Sitagliptin, respectively. Same was done for Metformin and Saxagliptin.

**d. Sample preparation:** 20 tablets were weighed and powdered and the quantity equivalent to one tablet transferred into a 100ml volumetric flask and made up with diluents and labeled as Sample stock solution. Sample stock solution was filtered by HPLC filters. 1ml of filtered sample stock solution was transferred to 100ml volumetric flask and made up with diluents.

**2. Chromatographic conditions:**

The chromatographic separation was achieved by injecting a volume of 10µl of standard into BDS (250mm x 4.6 mm, 5□). The mobile phase of composition Buffer and Acetonitrile taken in the ratio 73:27A were allowed to flow through the column at a flow rate of 1.2 ml/min for a period of 7 minutes at a wavelength of 212nm. The retention times (RT) were found at 2.4 and 3.0 minutes for Metformin & Sitagliptin respectively.

**3. Method Validation:** System suitability, Specificity, linearity, accuracy, LOQ, LOD were evaluated.

**Table 1:** Specificity data of Metformin and Sitagliptin

| | Average standard area | Standard deviation | RSD% |
|---|---|---|---|
| **Metformin** | 1861178 | 28996.31 | 1.56 |
| **Sitagliptin** | 439074 | 2429.53 | 0.55 |

**Table 2:** Specificity data of Metformin and Saxagliptin

| | Average standard area | Standard deviation | RSD% |
|---|---|---|---|
| **Metformin** | 11405013 | 170713.8 | 1.50 |

| | | | |
|---|---|---|---|
| **Saxagliptin** | 336239 | 3785.5 | 1.1 |

**Fig 4:** Optimized chromatogram of Metformin and Sitagliptin

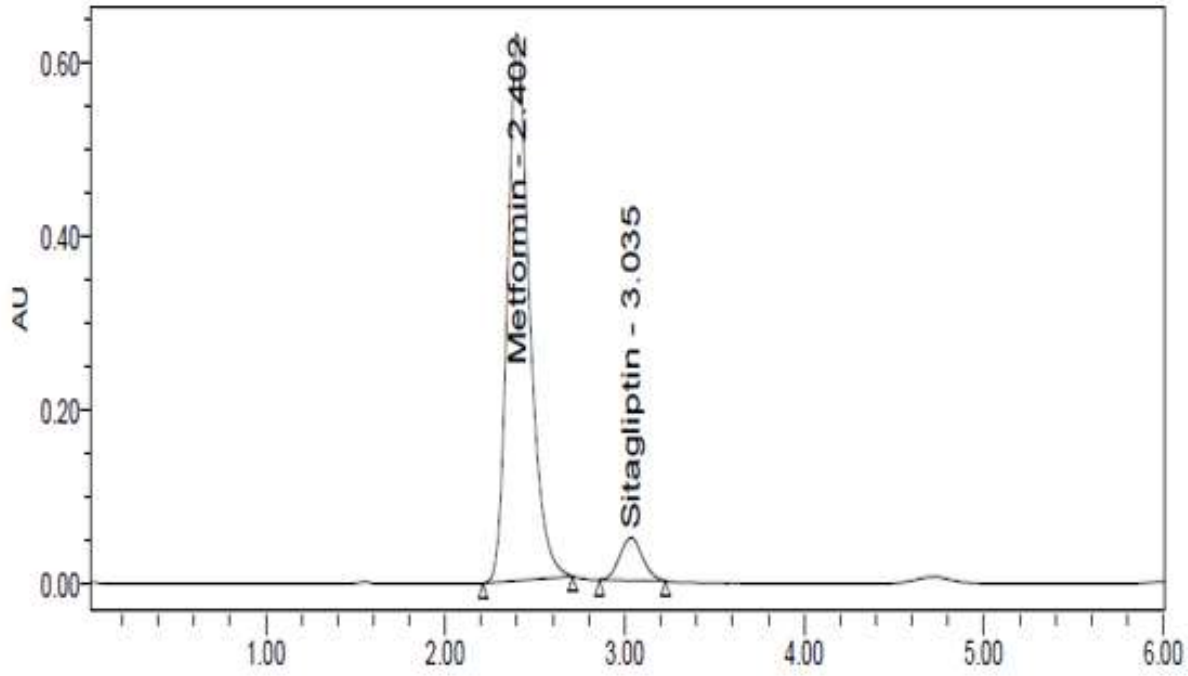


**Table 3:** Optimized chromatographic conditions of Metformin & Sitagliptin

| S. No. | Parameters | Conditions |
|---|---|---|
| 1 | Mobile phases | Buffer and Acetonitrile taken in the ratio 73:27A |
| 2 | pH | 3.3 |
| 3 | Column, make | BDS column (4.6 x 250mm, |
| 4 | Column temperature | 30°C |
| 5 | Injection | 10µl |
| 6 | Wave length | 212nm |
| 7 | Flow rates | 1.2ml/min |
| 8 | Run times | 07 min |
| 9 | Retention time (Metformin) | 2.4 min |
| 10 | Retention time (Sitagliptin) | 3.0 min |

**Fig 5:** Optimized chromatogram of Metformin and Saxagliptin

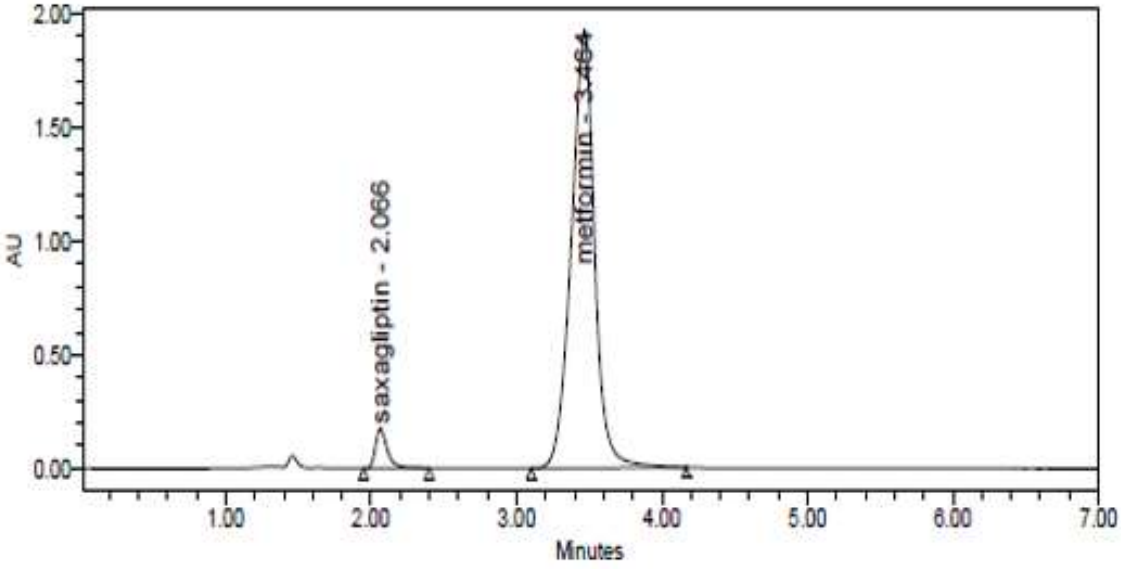



**Table 4:** Optimized chromatographic conditions of Metformin & Saxagliptin

| S. No | Parameter | Condition |
|---|---|---|
| i | Mobile phase | Buffer and Acetonitrile 42 : 58A |
| ii | pH | 3.3 |
| iii | Column, make | HYBER 150mm x 4.6 |
| iv | Column | 30°C |
| v | Injection | 10µl |
| vi | Wave length | 233nm |
| vii | Flow rate | 1.0ml/min |
| viii | Run time | 07 min |
| ix | Retention time | 3.5 min |
| x | Retention time | 2.0 min |

**Table 5:** Repeatability of Metformin & Sitagliptin

| | Metformin | Sitagliptin |
|---|---|---|
| **Repeatability (RSD%)** | 0.25 | 0.68 |

**Table 6:** Repeatability of Metformin and Saxagliptin

| | Metformin | Saxagliptin |
|---|---|---|
| **Repeatability (RSD%)** | 1.50 | 1.1 |

**Table 7:** Recovery data for Metformin & Sitagliptin.

| | Metformin | Sitagliptin |
|---|---|---|
| **% Recovered (RSD%)** | 1.75 | 1.22 |
| **% Recovery** | 100.42 | 100.15 |

**Table 8:** Recovery data for Metformin and Saxagliptin.

| | **Metformin** | **Saxagliptin** |
|---|---|---|
| **% Recovered (RSD%)** | 1.94 | 1.15 |
| **% Recovery** | 100.22 | 100.47% |

**Table 9:** Results of LOD and LOQ of Metformin & Sitagliptin

| | LOD ($\mu$ g/mL) | LOQ ($\mu$ g/mL) |
|---|---|---|
| **Metformin** | 0.25 | 0.75 |
| **Sitagliptin** | 0.01 | 0.03 |

**Table 10:** Results of LOD and LOQ of Metformin and Saxagliptin

| | LOD ($\mu$ g/mL) | LOQ ($\mu$ g/mL) |
|---|---|---|
| **Metformin** | 0.46 | 1.38 |
| **Saxagliptin** | 0.04 | 0.12 |

**Fig 6:** Linearity plot of Metformin & Sitagliptin

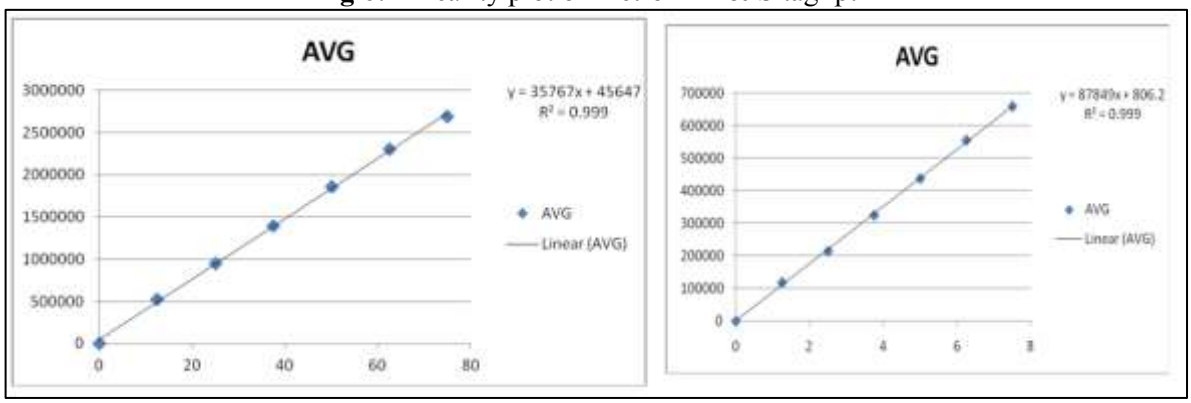



**Fig 7:** Linearity plot of Metformin & Saxagliptin

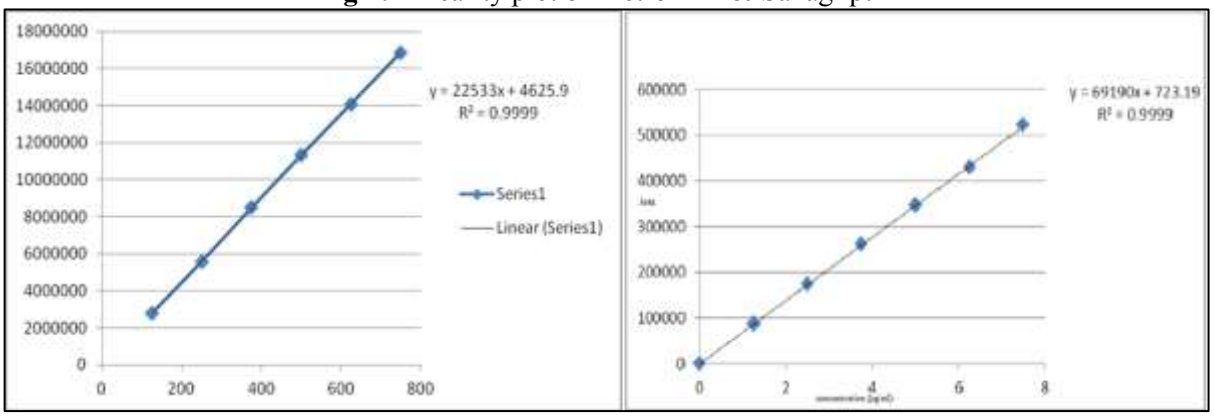



**Table 11**: System suitability parameters for Metformin & Sitagliptin

| **Parameters** | **Metformin** | **Sitagliptin** |
|---|---|---|
| **Retention times** | 2.4min | 3.0 min |
| **Theoretical plates** | 2879 | 2853 |
| **USP tailing** | 1.31 | 1.08 |

**Table 12:** System suitability parameters for Metformin & Saxagliptin

| **Parameters** | **Metformin** | **Saxagliptin** |
|---|---|---|
| **Retention times** | 3.5min | 2.0min |
| **Theoretical plates** | 2199 | 3186 |
| **USP tailing** | 1.1 | 1.45 |

**Conclusion:**

The retention times of Metformin & Sitagliptin were found to be 2.4min & 3.0 min, respectively. Here, number of theoretical plates were 2879 and 2853,USP tailing were 1.31,1.08 for Metformin & Sitagliptin, respectively; this showed optimized method met the system suitability parameters. The percentage mean recovery of Metformin & Sitagliptin were found to be 100.42, and 100.15%. The lowest values of LOD and LOQ were 0.25 and0.75µg/ml; 0.01 and 0.03µg/ml for Metformin & Sitagliptin, respectively.

The retention times of Metformin & Saxagliptin were found to be 3.5min & 2.0min, respectively. Number of theoretical plates were 2199 and 3186, USP tailing were 1.1, 1.45 for Metformin & Saxagliptin, respectively; this showed optimized method met the system suitability parameters. The percentage mean recovery of Metformin & Saxagliptin were found to be 100.22, and 100.47%, respectively. The lowest values of LOD and LOQ were 0.46 and 1.38µg/ml; 0.04 and 0.12µg/ml for Metformin & Saxagliptin, respectively.

**References:**


1. Awasthi, Ankit & Kumar, Ankit & Kumar, Rajan & Vishwas, Sukriti & Khursheed, Rubiya & Kaur, Jaskiran & Corrie, Leander & Kumar, Bimlesh & Gulati, Monica & Kumar, Deepak & Kaushik, Monika & Gupta, Gaurav & Prasher, Parteek & Kumar Chellappan, Dinesh & Kumar, Avvaru Praveen & Dua, Kamal & Singh, Sachin. (2022). RP-HPLC method development and validation for simultaneous estimation of mesalamine and curcumin in bulk form as well as nanostructured lipid carriers. South African Journal of Botany. 151.10.1016/j .sajb.2022.0 5.044.
2. Bhoir, Suvarna & Gaikwad, Poonam & Parab, Lavu & Shringarpure, Rohan & Savant, Sudha & Verma, Pratibha. (2009). RP-HPLC Method Development and Validation for the Simultaneous Estimation of Satranidazole and Ofloxacin in Pharmaceutical Dosage Form. Journal of chromatographic science. 49. 10.1093/chrsci/49.1.84.
3. Gangishetty, Sadana & Verma, Surajpal. (2013). RP-HPLC Method Development and Validation for Simultaneous Estimation of Clarithromycin and Paracetamol. ISRN Analytical Chemistry. 2013. 10.1155/2013/948547.
4. Gholve, Sachin & Gangapure, Sharddha & Birajdar, Mahesh & Mujewar, Imran & Bhusnure, Dr. Omprakash. (2019). RP-HPLC Method Development and Validation for Determination of Didanosine in Pharmaceutical Dosage Forms. Journal of Drug Delivery and Therapeutics. 9. 343-347. 10.22270/jddt.v9i4-s.3328.
5. Jagtap, Prajakta & Mahajan, Namrata & Parte, Anjali & Pananchery, Jeeja & Jain, Ashish. (2021). RP-HPLC Method Development and Validation for Simultaneous Estimation of Thymoquinone and Curcumin in Dosage form. Journal of Pharmaceutical Research International. 53-62. 10.9734/jpri/2021/v33i 59 B34352.
6. Kavitha, J. & Nagarajan, J.S.K. & Muralidharan, S. & Suresh, Bhavana. (2011). Development and validation of RP-HPLC method for simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablets: It's application to routine quality control analysis. International Journal of Pharmacy and Pharmaceutical Sciences. 3. 113-115.
7. Malothu, Narender & Senagashetty, Durga & Katamaneni, Padmalatha. (2018). RP-HPLC Method Development and Validation For Simultaneous Estimation of Paracetamol and Alprazolam in Bulk and Pharmaceutical Dosage Forms. American Journal of Pharm Tech Research. 8.282-293.10.46624/ajptr.2018.v8. i4.023.
8. Patel, Bhoomi & Bhavya, Mehta & Chaudhary, Ankit. (2020). Method development and validation for simultaneous estimation of lamivudine and zidovudine in tablet by reverse-phase high-performance liquid chromatography. Asian Journal of Pharmaceutical and Clinical Research. 73-77.10.22159/ajpcr.2020.v13i6.37 288.
9. Patel, Hetal & Damahe, Dulendra & Narkhede, Sachin. (2019). RP-HPLC Method Development and Validation for Simultaneous Estimation of Cilnidipine and Bisoprolol Fumarate in Tablet Dosage Form. International Journal of Chem Tech Research. 12. 269-276. 10.20902/I JCTR.2019.120130.
10. Sadasivam, Rubesh. (2017). RP-HPLC Method Development and Validation for Simultaneous Estimation of Linezolid and Cefixime in API and Pharmaceutical Dosage Form. 5. 10.21276/ijprhs.2017.04.03.
11. Sankarshana, T & Musthafa, Md. (2017). RP-HPLC method development and validation for simultaneous estimation of cobicistat and darunavir in bulk and pharmaceutical dosage form. International Journal of Medical Sciences and Pharma Research. 3. 12-22. 10.22270/ijmspr.v3i1.19.

12. Sathiyasundar, Ramalingam & Pandurangan, Perumal & Anupama, Sreelakshmi & Ramani, S & Nandini, Sai & Associate, M & Vidyaniketan, Sree. (2022). Robust RP-HPLC method development and validation of simultaneous estimation for selected anti-diabetic drug in pharmaceutical formulation. World Journal of Pharmaceutical Research. 11. 1964-1974. 10.20959/wjpr20223-23395.
13. Singh, Rahul & Pathak, Ashish & Chawla, Pooja. (2013). Method Development and Validation for Simultaneous Estimation of Ketorolac and Sparfloxacin by RP-HPLC. Indian Journal of Pharmaceutical and Biological Research. 1. 10.30750/ijpbr.1.4.17.
14. Taha, Elham & Fouad, Manal & Attia, Ali & Yousef, Mahmoud. (2019). RP-HPLC method development and validation for simultaneous estimation of ramipril and felodipine. European Journal of Chemistry. 10. 113-117. 10.5155/eurjchem.10.2.113-117.1832.
15. Venkateswara Rao, Battula & Vidyadhara, S. & Kumar, T. & Rao, D.V.. (2014). RP-HPLC method development and validation for the simultaneous estimation of metformin and sitagliptin in bulk and pharmaceutical formulations. Der Pharmacia Lettre. 6. 301-307.