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# Development And Validation Of RP-HPLC Method For Anagliptin And Metformin Hydrochloride And Its Related Impurities In Tablet Dosage Form

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**ABSTRACT**

**Introduction:** Anagliptin (ANA) and metformin HCl (MET) are key antidiabetic medications for managing type 2 diabetes by regulating blood glucose through different mechanisms. Despite various analytical methods, a stability-indicating RP-HPLC method for quantifying both drugs and their related impurities is lacking. This study develops and validates a precise, sensitive RP-HPLC method for determining anagliptin, metformin, and anagliptin impurity A, as accordance Q2 (R1) guidelines.

**Objectives:**Reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated for estimating the impurities of metformin HCl and anagliptin in a combined tablet dosage form.

**Methods:**The RP-HPLC analysis utilized a KROMASIL-$C_{18}$ column (250mm x 4.6mm, 5µm particle size), with a mobile phase of acetonitrile and 0.05M potassium dihydrogen phosphate buffer (pH 3.0) in a 50:50 ratio. The detection was carried out at 220 nm with a flow rate of 1.0 mL/min.This analytical method was validated according to the International Council for Harmonization (ICH) guidelines.

**Results**: The linearity of metformin HCl (25-75µg/ml) with LOQ 2.99µg/ml, anagliptin (5-15µg/ml) with LOQ 1.304µg/ml andanagliptin Impurity A (5-15µg/ml) with LOQ 1.063µg/ml and Limit of Detection was

found to be 0.43µg/ml for anagliptin,0.97µg/ml for Metformin HCl and 0.35µg/ml for impurity A. The correlation coefficient was more than 0.999 for Metformin HCl, anagliptin and Impurity A. The relative standard deviation value for repeatability, Interday precision and intraday precision was less than 2% which indicate the method is robust in nature.**Conclusion:**In summary, this RP-HPLC method is linear, sensitive, precise, accurate, and robust, making it highly suitable for the quantitative determination of metformin HCl, anagliptin, in presence of anagliptin impurity A in combined tablet dosage form.

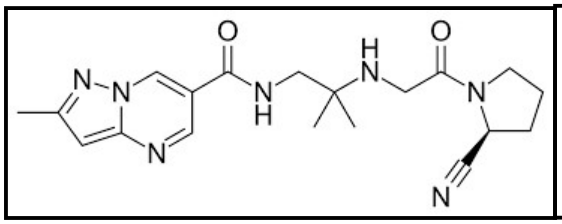
**Key word:** MetforminHCl, Anagliptin, Impurity, RP-HPLC Method, Validation

## INTRODUCTION

Antidiabetic medications are crucial for managing diabetes mellitus by lowering blood glucose levels. Type 2 diabetes mellitus presents with symptoms such as polyphagia (increased hunger), polyuria (frequent urination), and polydipsia (excessive thirst), requiring lifelong treatment with appropriate antidiabetic drugs. Anagliptin (ANA) is a Dipeptidyl peptidase 4 (DPP-4) inhibitor used in the treatment of type 2 diabetes mellitus. Chemically referred to as N-[2-[[2-[(2S)-2-Cyanopyrrolodin-1-yl]-2-oxoethyl] amino]-2-methylpropyl-2-methylpyrazolo [1, 5-a] pyrimidine-6-carboxamide, ANA prevents the breakdown of GLP-1, a hormone released after meals. By inhibiting GLP-1 degradation, ANA helps regulate insulin secretion from beta cells and reduces glucagon release from alpha cells, thereby maintaining stable blood glucose levels.Metformin Hcl (MET), a biguanide class antidiabetic drug, is widely recognized as a first-line treatment according to international guidelines. Chemically known as 3-(diaminomethylidene)-1, 1-dimethylguanidine, MET primarily reduces hepatic gluconeogenesis and decreases glucose production by the liver.Both medications play roles in managing type 2 diabetes mellitus by addressing different mechanisms to effectively lower blood glucose levels.

Impurity profiling encompasses a suite of analytical techniques designed to detect, identify, and quantify both known and unknown impurities, including organic and inorganic substances and residual solvents, in bulk drugs and pharmaceutical formulations. This comprehensive approach is essential for evaluating the quality, safety, efficacy, and stability of these substances, making it a fundamental aspect of modern drug analysis. During drug impurity profiling provides critical insights into the quality, safety, efficacy, and potential toxicity of drugs, while also establishing various limits of detection and quantification.

Although literature offers several advanced techniques for analyzing anagliptin and metformin HCl individually or in combination with other drugs, such as UV spectrometry, HPLC, and stability-indicating HPLC methods, there is currently a notable absence of a stability-indicating RP-HPLC method specifically for determining anagliptin and metformin HCl along with their related impurities in pharmaceutical dosage forms. Therefore, a precise, accurate, and sensitive stability-indicating chromatographic method was developed and validated in accordance with the Q2 (R1) guideline. This method aims to enable reliable quantification of anagliptin,metformin and anagliptin impurity A.



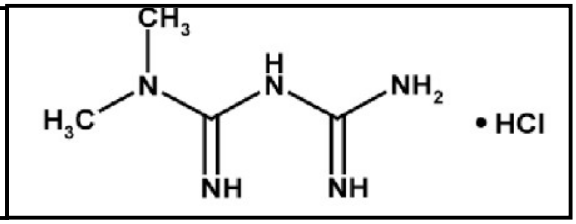




**Fig 1 AnagliptinFig 2 Metformin HCl**

## OBJECTIVES

### Materials

The API of anagliptin was obtained as gift sample from Intas Pharmaceutical Pvt Ltd., Ahmedabad. metformin HCl API was obtained as gift sample from nucleas lab, chtral. As well as impurity A was obtainedas gift sample from Intas Pharmaceutical Pvt Ltd., All solvent like HPLC grade water, methanol, ACN, were obtained from Merck specialties Pvt. Ltd.Mumbai, India.

**Instrumentation**

The method development utilized the following instruments: the Shimadzu LC-20 AT HPLC chromatographic system, a Shimadzu digital weighing balance (model ATX 224), a pH meter from Lab Scientific Pvt. Ltd, a Frontline Ultrasonic Cleaner ultrasonicator, a hot air oven from India, and a Thermolab unit from Mumbai. Filtration was performed using a 0.45µ Millipore filter.

**METHODS**

**Chromatographic Condition**

A chromatographic method was employed to separate anagliptin and metformin HCl using a KROMASIL-$C_{18}$ column (250mm x 4.6mm, 5µm particle size). The mobile phase consisted of Buffer pH3: Acetonitril (50:50 v/v) at a flow rate of 1 mL/min. Samples were injected at 20.0 µL and detection was conducted at a wavelength of 220 nm over a 15-minute runtime.

**Preparation of Mobile Phase**

The potassium dihydrogen phosphate was prepared by dissolving accurately weight 6.8gm of potassium dihydrogen phosphate in 1000ml HPLC grade water in a 1L volumetric flask and pH was adjusted to pH 3 with ortho phosphoric acid and mobile phase is prepared with the ratio of Acetonitrile 50 ml and Contained 50 ml of buffer pH 3 for sharpen the peak.

**Standard Solution Preparation**

**Preparation of standard stock and working standard stock solution of Anagliptin**

Weigh accurately 10mg of anagliptin transfer 10ml volumetric flask dissolved in acetonitrile and it make up to mark to get 1000µg/ml(1000ppm) of anagliptin standard solution. Pipette out 1ml of above solution transfer 10ml volumetric flask make up to mark with ACN to get 100µg/ml (100ppm).

**Preparation of standard and working standard stock solution of Metformin HCl**

Weighing accurately 10mg of metformin HCl in 10ml volumetric flask make up to the mark with Acetonirtil and sonicated for 10 min. to get 1000µg/ml (1000ppm) from this solution Pipette out 5ml from above solution transfer into 10ml of volumetric flask and make up to with solvent to get 500µg/ml (500ppm).

**Standard Stock Solution (1000ppm) and working standard solution of Anagliptin Impurity A (100ppm):**

Take 10mg anagliptin Impurity A into 10mL volumetric flask and dissolved into ACN make up to with ACN and to get 1000ppm. Transfer 1ml into 10mL volumetric flask from above solution and make up to with diluent and to get 100ppm.

**Preparation of Sample Solution from Pharmaceutical Marketed Tablets**

**Stock solution**

About 10 tablets of ANAMET were weighed, and an average weight of 10 tablets was determined and powdered finely in a mortar. Powdered tablet equivalent to 10 mg of Anagliptin and 50 mg of Metformin HCl was accurately weighed and transferred into 100 ml volumetric flask and diluted with Mobile phase up to the mark to get 100 mcg/ml of anagliptin and 500 mcg/ ml of metformin HCl.

**Working solution**

From above stock solution pipette out 1 ml and transferred in to 10 ml volumetric flask and diluted up to the mark with Mobile Phase to get 50 mcg/ml of metformin HCl and 10 mcg/ml of anagliptin

**Chromatographic Separation**

Standard solutions of metformin HCl and anagliptin, along with anagliptin impurity (Impurity A), were

administered into the column using a 20 µL micro-syringe. The chromatographic run was conducted for the necessary duration, with detection occurring at a wavelength of 220 nm. The chromatogram was terminated once complete separation was achieved. Data pertaining to resolution, retention time, and peak characteristics such as height and area were recorded using the Lab-solution software.

**Method Validation**

The proposed method was validated in accordance with ICH guidelines Q2 (R1)

Validation covered various parameters including accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness.

**Linearity**

Linearity is expressed in term of correlation co-efficient of linearity regression analysis. Calibration curve from working solution containing 500µg/mL MET 100µg/mL ANA and 100µg/mL Impurity A. aliquots 0.5, 0.75, 1, 1.25, 1.5mL were transferred in clean and dry 10mL vol. flask respectively and sonicated. The volume was made up to the mark with diluents. This yielded solution of 25, 37.5, 50, 62.5,75 µg/mL of MET and 5, 7.5, 10, and 12.5,15 µg/mL of ANA and 5, 7.5, 10, 12.5,15 µg/mL of Impurity A respectively. an aliquot(20 µl) of each

solution was injected under the operating chromatographic condition as described earlier calibration curve was prepared by plotting peak areas versus concentration.

**LOD and LOQ**

The Limits of Detection (LOD) and Limits of Quantification (LOQ) for both the drugs and impurity were determined based on the data obtained from linearity studies. Subsequently, the LOQ and LOD were computed using the following formula:

LOQ = 10*Standard Deviation/Slope of Calibration curve

LOQ= 3.3*Standard Deviation/Slope of Calibration curve

**Precision**

System precision was evaluated by performing six injections of a standard solution containing Metformin HCl (500µg/mL), along with anagliptin (100µg/mL) and its Impurity A (100µg/mL). The resulting chromatograms were analysed and peak areas recorded to assess repeatability.For precision testing, a standard solution with concentrations of metformin HCl 500 µg/mL, anagliptin 100 µg/mL and its Impurity A 100 µg/mLwere used. These solutions were tested for Interday precision by analyzing them on different days, and for Intraday precision by analyzing them multiple times on the same day. From these tests, the percentage relative standard deviation (%RSD) was calculated to determine the precision of the method.

**Accuracy**

Accuracy was determined by calculating recovery of ANA, METand ANA. Impurity A by the standard addition method known amounts of standard solution to pre-analyzed samples. Each solution was injected in triplet who is in accordance with ICH guideline which proves method to be accurate.

**Robustness**

The robustness study was conducted under chromatographic conditions to assess the impact of minor variations as outlined in the Chromatographic Conditions section. This study focused on factors that were identified as critical sources of variability in the operating procedures. Specifically, adjustments included altering the mobile phase ratio by ±2 mL, pH of mobile phase ±0.2and modifying the flow rate of the mobile phase by ±0.2 mL/min. throughout these experiments, the composition of the mobile-phase components remained unchanged. The effects of these alterations were then evaluated in terms of their impact on the system suitability for standard preparation

**RESULTS**

After conducting several trials, the optimal chromatographic conditions were established as follows:

**Table 1 Optimized Chromatoraphic Condition**

| Parameters | Conditions |
|---|---|
| Mobile Phase | Acetonitril : buffer pH 3 50:50 v/v |
| Stationary Phase | Kromasil $C_{18}$ 5µ (250mm x 4.6mm, 5µm particle size) |
| Flow rate | 1 ml/min |
| Run time | 15 min |
| Volume of injection | 20 mL |
| Detection of wavelength | 220 nm |

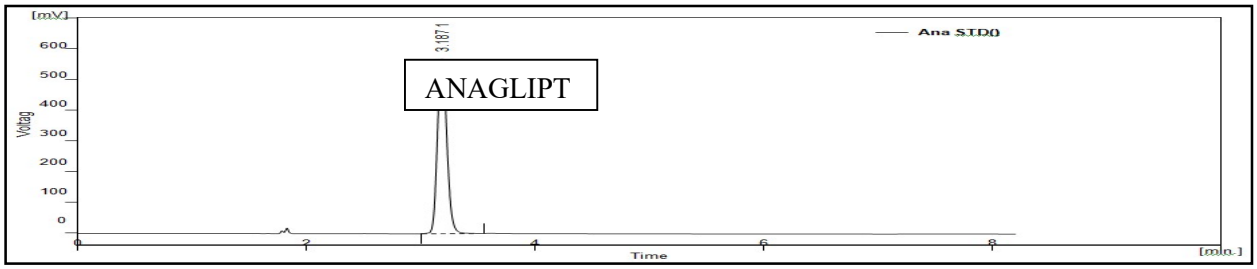



**Figure 3 Standard Chromatogram of Anagliptin**

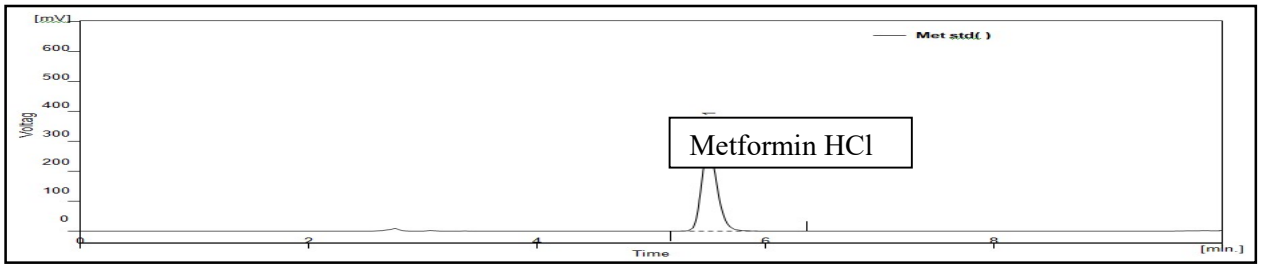



**Figure 4 Standard Chromatogram of Metformin HCl**

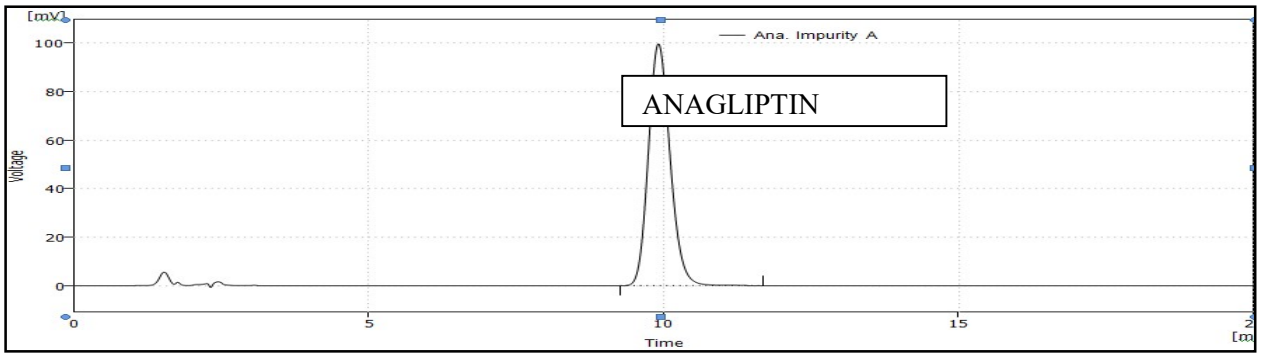



**Figure 5 Standard Chromatogram of Anagliptin Impurity A**

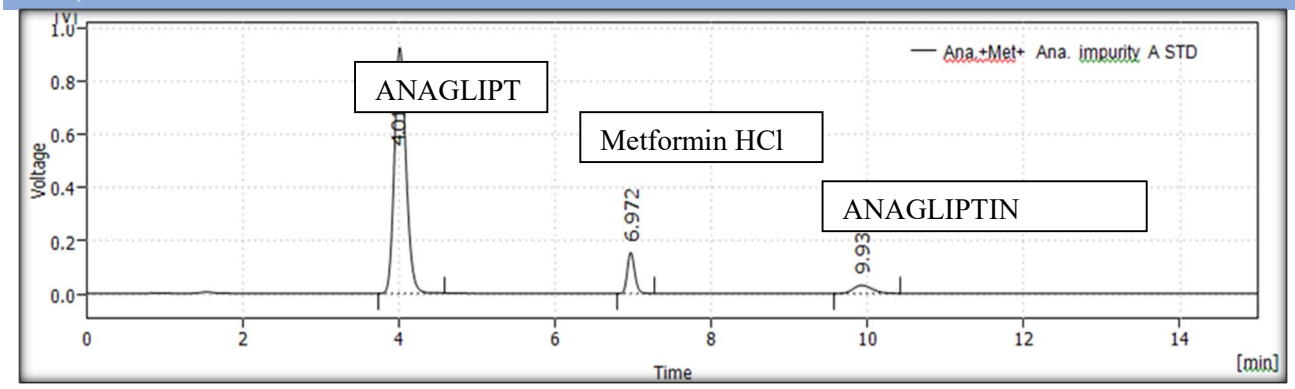



**Figure 6 Optimized Chromatogram of Anagliptin and Metformin HCl &Ana. Impurity A**

**System Suitability Parameter**

System Suitability was assessed using various parameters such as retention time, theoretical plates, resolution, and tailing factor. The evaluation aimed to ensure the system's repeatability and resolution was sufficient for the intended analysis. The recorded system suitability parameters for Anagliptin, Metformin HCl, and impurity A are detailed below:

**Table 2 System Suitability Parameter**

| Parameters | Anagliptin | Metformin HCl | Impurity A |
|---|---|---|---|
| Retention time | 4.011 | 6.972 | 9.913 |
| Theoretical plates | 3409 | 8739 | 3513 |
| Tailing factor | 1.255 | 1.242 | 1.269 |
| Resolution | -- | 13.13 | - |

**Specificity**

Specificity was confirmed by analyzing the resolution factor between drug peaks and their closest resolving peaks, as well as among all other peaks. Method specificity was evaluated by comparing chromatograms of the standard and test solutions to ensure there were no interfering peaks with the analyte peak.

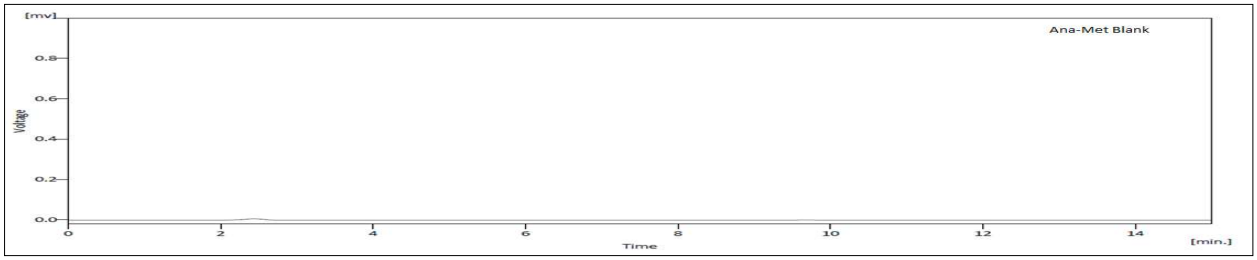



**Figure 7 BlankChromatogram of Anagliptin, Metformin HCl, Anagliptin Impurity A**

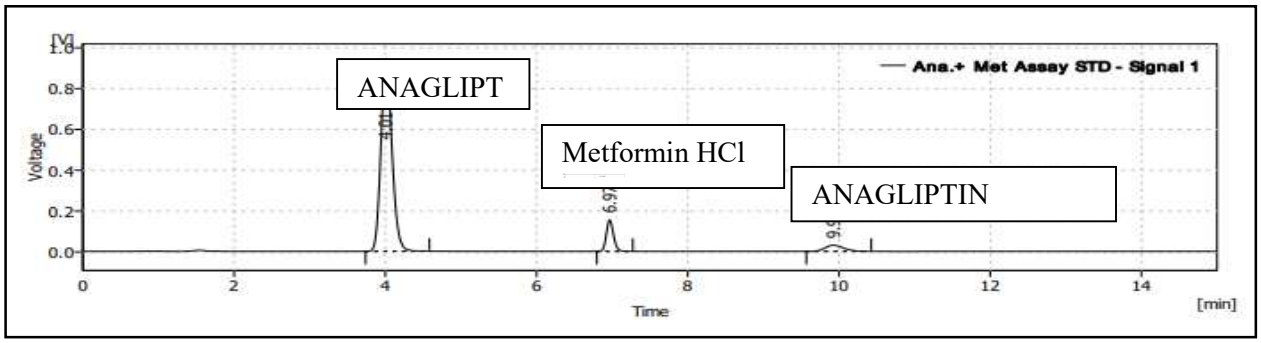


**Figure 8 Standard Chromatogram of Anagliptin, Metformin HCl, Anagliptin Impurity A**

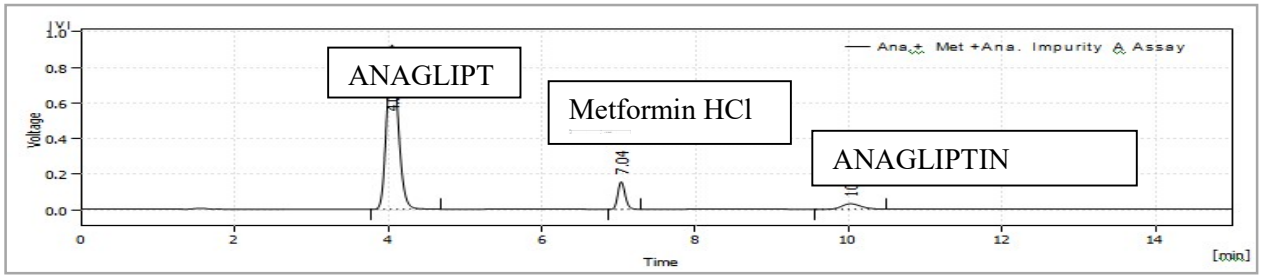



**Figure 9Sample Chromatogram of Anagliptin, Metformin HCl, Anagliptin Impurity A**

**Linearity**

The linearity of anagliptin, metformin HCl and Impurity A was assessed by analyzing a combined standard solution within specified ranges: 25 to 75 µg/mL for metformin HCl and 5 to 15 µg/mL for both anagliptin and anagliptin Impurity A. The correlation coefficient (r) for the calibration curves of Anagliptin, Metformin HCl, and Impurity A was found to be ≥ 0.999 for each compound.

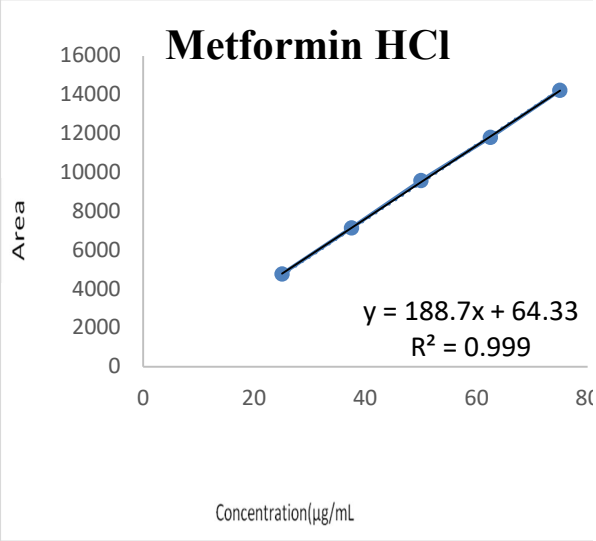



**Figure 10 Calibration Curve of METHCL**

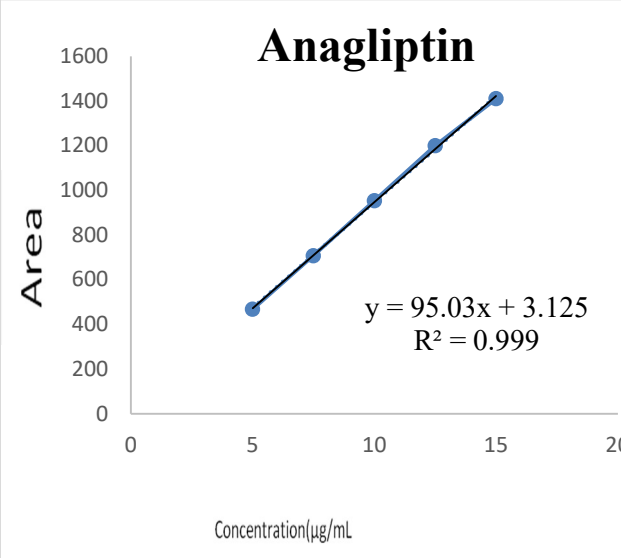



**Figure 11Calibration Curve of Anagliptin**

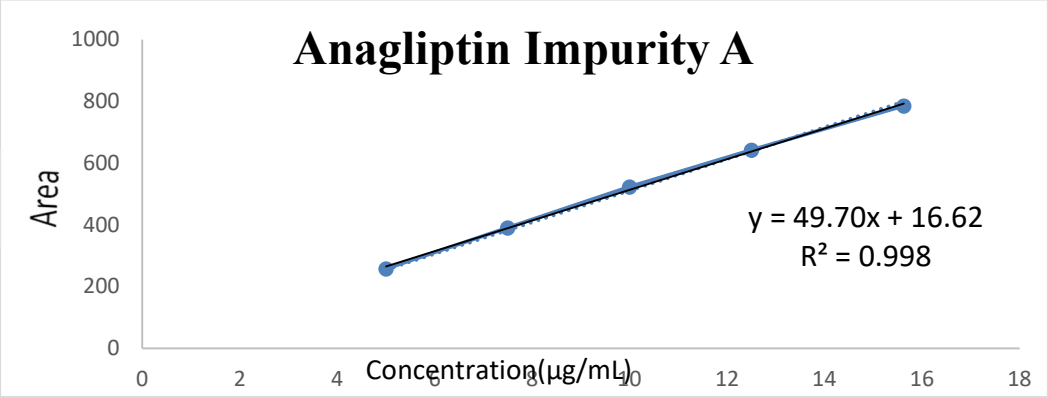



**Figure 12Calibration Curve of Anagliptin Impurity A**

**Table 3 Analytical data of linearity**

| Metformin HCl | | Anagliptin | | Anagliptin Impurity A | |
|---|---|---|---|---|---|
| **Concentration (µg/ml)** | **Area± S. D (n=6)** | **Concentration (µg/ml)** | **Area ± S. D (n=6)** | **Concentration (µg/ml)** | **Area± S. D** |
| 25 | 4767.7±6.651 | 5 | 467.9±1.10 | 5.0 | 257.9±2.05 |
| 37.5 | 7133.4±13.28 | 7.5 | 706.2±3.54 | 7.5 | 390.6 ±3.09 |
| 50 | 9574±19.33 | 10 | 953.1±7.22 | 10.00 | 522.8±8.10 |
| 62.5 | 11799.9± 21.60 | 12.5 | 1199.7±9.84 | 12.5 | 642.5±10.11 |
| 75 | 14230.6± 28.81 | 15 | 1409.1±16.15 | 15 | 785.3±14.71 |
| **Correlation Coefficient (r)** | 0.999 | **Correlation Coefficient (r)** | 0.999 | **Correlation Coefficient (r)** | 0.998 |

**Precision**

**Repeatability**

The repeatability data for peak area measurement of Metformin HCl, Anagliptin and Impurity A was assessed based on six measurements of the same solution. The mean peak area observed was 9546.65 for Metformin HCL, 1006.79 for Anagliptin and 518.17 for Anagliptin Impurity A with % RSD 0.172, 0.171 and 0.173. The % RSD values observed within the acceptance limit of NMT 2% results shown in table 4

**Table: 4 Repeatability precision data for Estimation of ANA, MET&ANA. Impurity A**

| MetforminHCl | | | | | Anagliptin | | | | Anagliptin Impurity A | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sr no.** | **Conc. (µg/ml)** | **Area** | **Mean ± S. D (n=6)** | **% RSD** | **Conc. (µg/ml)** | **Area** | **Mean ± S. D (n=6)** | **% RSD** | **Conc. (µg/ml)** | **Area** | **Mean ± S. D (n=6)** | **% RSD** |
| 1 | 50 | 9534.63 | 9546.65± 16.42 | 0.172 | | 1005.54 | 1006.79± 1.734 | 0.171 | | 517.53 | | |
| 2 | | 9526.56 | | | | 1004.73 | | | | 517.114 | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | | 9559.18 | | | 1008.13 | | | | 518.863 | 518.1±0.89 | 0.173 |
| 4 | | 9540.12 | | 10 | 1006.12 | | | 10 | 517.828 | | |
| 5 | | 9571.15 | | | 1009.44 | | | | 519.534 | | |
| 6 | | 9546.35 | | | 1006.79 | | | | 518.173 | | |

| MetforminHCl | | | | Anagliptin | | | Anagliptin Impurity A | | |
|---|---|---|---|---|---|---|---|---|---|
| Sr no. | Conc. (µg/ml) | Area Mean ± S. D (n=3) | % RSD | Conc. (µg/ml) | Area Mean ± S. D (n=3) | % RSD | Conc. (µg/ml) | Area Mean ± S. D (n=6) | % RSD |
| 1 | 25 | 4820.68±1.20 | 1.201 | 5 | 515.64±6.19 | 1.201 | 5 | 266.89±3.18 | 1.179 |
| 2 | 50 | 9472.29±37.96 | 0.401 | 10 | 1002.63±1.69 | 0.169 | 10 | 519.88±1.47 | 0.282 |
| 3 | 75 | 14393.69±85.27 | 0.592 | 15 | 1526.71±9.10 | 0.596 | 15 | 790.49±4.704 | 0.595 |

**Interday and intraday precision**

The data for Interdayand Intraday precision for metforminHCL ,anagliptin and anagliptin Impurity A is shown in below table 5 & 6. The %RSD for Metformin, Anagliptin and Anagliptin Impurity A was found below in the given table 5 & 6 respectively.

**Table 5 Analytical Data for Intraday**

| MetforminHCl | | | | Anagliptin | | | Anagliptin Impurity A | | |
|---|---|---|---|---|---|---|---|---|---|
| Sr no. | Conc. (µg/ml) | Area Mean ± S. D (n=3) | % RSD | Conc. (µg/ml) | Area Mean ± S. D (n=3) | % RSD | Conc. (µg/ml) | Area Mean ± S. D (n=6) | % RSD |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 25 | 4820.68±1.20 | 1.201 | 5 | 515.64±6.19 | 1.201 | 5 | 266.89±3.18 | 1.179 |
| 2 | 50 | 9472.29±37.96 | 0.401 | 10 | 1002.63±1.69 | 0.169 | 10 | 519.88±1.47 | 0.282 |
| 3 | 75 | 14393.69±85.27 | 0.592 | 15 | 1526.71±9.10 | 0.596 | 15 | 790.49±4.704 | 0.595 |

**Table 6 Analytical Data for Interday**

| **MetforminHCl** | | | | **Anagliptin** | | | **Ana. Impurity A** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sr no.** | **Conc. (µg/ml)** | **Area Mean ± S. D (n=3)** | **% RSD** | **Conc. (µg/ml)** | **Area Mean ± S. D (n=3)** | **% RSD** | **Conc. (µg/ml)** | **Area Mean ± S. D (n=6)** | **% RSD** |
| 1 | 25 | 4983.43±34.22 | 0.687 | 5 | 528.55±3.63 | 0.687 | 5 | 273.42±1.88 | 0.689 |
| 2 | 50 | 9513.07±15.16 | 0.159 | 10 | 1008.10±2.09 | 0.207 | 10 | 522.39±0.96 | 0.183 |
| 3 | 75 | 14581.03±18.21 | 0.125 | 15 | 1546.55±1.93 | 0.125 | 15 | 800.26±0.96 | 0.12 |

**LOD and LOQ**

The limit of Detection (LOD) and limit of quantitation (LOQ) were obtained by calculating using the standard formula as per the ICH guidelines, LOD=3.3(σ/S), LOQ=10(σ/S) Where σ is Standard deviation of the response and S is slope of the calibration curve. The LOD and LOQ for the drugs were estimated using the linearity data (figure 5and6) repeated calibration curve 5 time and calculated deviation of the intercepts. The LOD for ANA was observed 0.430µg/mL, MET 0.97µg/mL and 0.351µg/mL for anagliptin impurity A. The LOQ for ANA was observed 1.304µg/mL, for MET 2.99µg/mL and 1.063µg/mL for anagliptin impurity A the results were show in table 7.

**Table 7 LOD and LOQ data of ANA, MET and Impurity A**

| **Drugs Name** | **LOD** | **LOQ** |
|---|---|---|
| **METFORMIN HCl** | 0.97µg/mL | 2.99µg/mL |
| **ANAGLIPTIN** | 0.430µg/mL | 1.304µg/mL |

| | | |
|---|---|---|
| **ANA. Impurity A** | 0.351µg/mL | 1.063µg/mL |

**Accuracy**

Accuracy was determined by calculating recovery of MET, ANA and ANA. Impurity A by the standard addition method known amounts of standard solution to pre-analyzed samples. Each solution was injected in triplet and recoveries were in between 100.3-101.7% for MET, 100.1-100.3% for ANA and 100.2-100.8% for ANA. Impurity A which is in accorandance with ICH guideline which prove method to be accurate. (Table 8)

**Table 8 Analytical data for Accuracy**

| DRUG | %Level | Amount of sample (mg) | Amount of standard (mg) | %Recovery ± SD | %RSD |
|---|---|---|---|---|---|
| **Metformin HCl** | 80 | 40 | 40.67 | 101.7 ± 0.31 | 0.427 |
| | 100 | 50 | 50.15 | 100.30 ± 0.25 | 0.391 |
| | 120 | 60 | 60.97 | 101.61±0.30 | 0.616 |
| **Anagliptin** | 80 | 8 | 8.09 | 101.13± 0.52 | 0.420 |
| | 100 | 10 | 10.01 | 100.1± 0.04 | 0.384 |
| | 120 | 12 | 12.03 | 100.3± 0.13 | 0.613 |
| **ANA.Imurity A** | 80 | 8 | 8.08 | 101.01± 0.07 | 0.364 |
| | 100 | 10 | 10.02 | 100.2± 0.38 | 0.282 |
| | 120 | 12 | 12.09 | 100.8± 0.18 | 0.520 |

**Robustness**

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing three small changes.

- ❑ Change flow rate by 10%. (i.e. 0.8ml/min and 1.2 ml/min)
- ❑ Change in mobile phase by 0.2 unit
- ❑ Change in pH by 0.2unit (i.e. pH 3.2 and pH 2.8)

After each sample solution was injected and peak area, tailing factor and retention time were checked. The results are shown in the table 8. Variation seen was within the acceptable range respect to peak asymmetry and theoretical plates, so the method was found to be robust.

**Table 9 Robustness Data of METFORMIN HCl, ANAGLIPTIN and Impurity A**

| METFORMIN HCl | | | | | | |
|---|---|---|---|---|---|---|
| **Sr no.** | **Area at Flow rate(+0.2 ml/min) ±SD (n=3)** | **Area at Flow rate(+0.2 ml/min) ±SD (n=3)** | **Area at pH(+0.2 ml/min) ±SD (n=3)** | **Area atpH (-0.2) ±SD (n=3)** | **Area atMobile phase(+2)±SD** | **Areaat Mobile phase(-2)±SD (n=3)** |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | **(n=3)** | |
| 1 | 9529.49±1.1 | 9530.53±2.13 | 9532.18±0.89 | 9530.62±2.5 | 9532.69±0.67 | 9530.45±1.28 |
| 2 | 9528.42±1.8 | 9528.12±1.91 | 9528.12±1.3 | 9530.48±1.1 | 9531.62±0.89 | 9531.695±1.12 |
| 3 | 9530.62±1.5 | 9531.26±1..82 | 9531.26±2.1 | 9532.45±1.8 | 9530.14±0.94 | 9530.14±1.21 |
| %R.S.D | 0.012 | 0.022 | 0.013 | 0.008 | 0.013 | 0.008 |
| **ANAGLIPTIN** | | | | | | |
| **Sr no.** | **Area at Flow rate(+0.2 ml/min) ±SD (n=3)** | **Area atFlow rate(-0.2ml/min)±SD (n=3)** | **Area atpH (+0.2) ±SD (n=3)** | **Area at pH (-0.2)±SD (n=3)** | **Area atMobile phase(+2)±SD (n=3)** | **Area at Mobile phase(-2)±SD (n=3)** |
| 1 | 1010.43±0.72 | 1010.12±1.08 | 1008.15±1.9 | 1010.52±0.79 | 1011.23±1.20 | 1010.12±0.69 |
| 2 | 1012.12±1.23 | 1015.18±1.71 | 1010.25±3.8 | 1010.16±1.12 | 1012.58±7.65 | 1009.98±1.21 |
| 3 | 1010.05±0.89 | 1007.36±0.67 | 1010.84±2.4 | 1009.65±1.33 | 1014.57±1.03 | 1010.53±0.96 |
| %R.S.D | 0.109 | 0.392 | 0.166 | 0.028 | 0.140 | 0.044 |
| **ANAGLIPTIN Impurity A** | | | | | | |

| Sr no. | Area at Flow rate(+0.2 ml/min) ±SD (n=3) | Area at Flow rate(-0.2ml/min) ±SD (n=3) | Area at pH (+0.2) ±SD (n=3) | Area at pH (-0.2)±SD (n=3) | Area atMobile phase(+2)±SD (n=3) | Area at Mobile phase(-2)±SD (n=) |
|---|---|---|---|---|---|---|
| 1 | 523.30±0.29 | 524.89±0.39 | 526.43±0.46 | 522.21±1.36 | 521.45±1.89 | 523.64±1.81 |
| 2 | 526.12±0.67 | 521.69±0.85 | 524.13±0.94 | 524.61±1.29 | 522.68±1.63 | 524.12±2.01 |
| 3 | 524.61±1.19 | 524.36±2.31 | 522.11±1.02 | 523.89±2.03 | 523.89±0.96 | 524.62±1.06 |
| %R.S.D | 0.269 | 0.328 | 0.233 | 0.094 | 0.413 | 0.235 |

**Table 9 Summary of Validation data**

| Parameter | | Metformin HCl | Anagliptin | Anagliptin Impurity A |
|---|---|---|---|---|
| Linearity (Regrassion Value) | | 25-75 µg/ml | 5-15 µg/ml | 5-15 µg/ml |
| Repeatability(%RSD, n=6) | | 0.172 | 0.171 | 0.173 |
| Precision (%RSD) | Intra-day (n=3) | 1.201 to 0.592 | 1.201 to 0.596 | 1.179 to 0.595 |
| | Inter-day (n=3) | 0.125 to 0.687 | 0.125 to 0.687 | 0.120 to 0.689 |
| Limit of Detection | | 2.99µg/ml | 1.304 µg/ml | 1.063µg/ml |
| Limit of Quantification | | 0.97µg/ml | 0.430µg/ml | 0.351µg/ml |
| Robustness | | Robust | Robust | Robust |

**DISCCUSION**

There is currently no analytical method in the literature for the determination of related impurities of Metformin HCl and anagliptin using RP-HPLC. This study aims to developing and validating such a method. The RP-HPLC method described here is specific, sensitive, rapid, and straightforward to execute, enabling simultaneous estimation of metformin Hcl, anagliptin, and their related impurities. The method effectively

separates and resolves chromatographic peaks of these compounds. The mobile phase used consisted of ACN and buffer pH 3 in a 50:50 v/v ratio. Recoveries from all formulations matched their label claims, indicating no interference from formulation excipients. Validation according to ICH guidelines confirmed the method's specificity, precision, linearity, and robustness. In conclusion, this RP-HPLC method is suitable for routine analysis of related impurities of metformin HCl and anagliptin in combined dosage forms.

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