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## Simultaneous estimation of vildagliptin and metformin hydrochloride by using RP-HPLC in bulk and pharmaceutical dosage form

D Raju, P Karunakar, China Babu Jonnakuti and N Asha

#### Abstract

The estimation of Metformin hydrochloride and Vildagliptin by simultaneous method using RP-HPLC method was performed by using Chromosil ODS C<sub>18</sub> column having 250 x 4.6mm 5 $\mu$ , and mobile phase containing 0.1M Di Potassium hydrogen phosphate and Methanol at the ratio (60:40% v/v) adjust the pH:9.2 by using Ortho phosphoric acid. The flow rate was 0.5ml/min and detection was observed at 258nm. The retention time of Metformin and Vildagliptin was 1.43min and 5.32min respectively. The all validation parameters were accepted within the limits as per ICH guidelines. The specificity was performed under stress conditions like acid, base, peroxide, light and heat and observed the degradation of drugs. It was found to be within the acceptance limit. Hence it was concluded that the developed method was found to be simple, precise, accurate and rapid method for the routine analysis of Vildagliptin and Metformin hydrochloride API and pharmaceutical dosage form in laboratories.

**Keywords:** metformin hydrochloride, vildagliptin, RP-HPLC method

#### Introduction

Chromatography is a technique by which the components in a sample, carried by the liquid or gaseous phase, are resolved by sorption-desorption steps on the stationary phase [1-3].

#### Types of HPLC techniques [4-8]

The types of chromatography based on following parameters include

##### Based on modes of chromatography

- Reversed phase chromatography
- Normal phase chromatograph

##### Based on principle of separation

- Adsorption chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

##### Based on elution technique

- Isocratic separation
- Gradient separation

##### Based on the scale of operation

- Analytical HPLC
- Preparative HPLC

#### High performance liquid chromatography [9-16]

High Performance Liquid Chromatography (HPLC) is one of the chromatography techniques, It is most widely used analytical method. HPLC utilizes a liquid mobile phase to separate the components of a mixture. These components (or analytes) are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure.

In the column, the mixture is resolved into its individual components. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases.

**2. Materials and equipments used**

Metformin, Vildagliptin, Di Potassium Hydrogen Phosphate, Methanol, Chromosil C<sub>18</sub> column, HPLC.

**3. Results and Discussions**

**Table 1:** Optimized Chromatographic conditions for galvusmet

|                  |   |
|------------------|---|
| Mobile phase     | Di Potassium Hydrogen Phosphate:Methanol 60:40(v/v)   |
| Detector         | Photo Diode Array (PDA)                               |
| Pump mode        | Isocratic   |
| Ph               | 9.2   |
| Diluents         | Mobile phase  |
| Column           | Chromosil C <sub>18</sub> column (250mm X 4.6 mm, 5μ) |
| Column Temp      | 35°C  |
| Wavelength       | 258nm   |
| Injection Volume | 10 μl   |
| Flow rate        | 0.5 ml/min  |
| Run time         | 7 minutes   |
| Retention Time   | 1.43min for Metformin and 5.32min for Vildagliptin    |

**Optimized method**

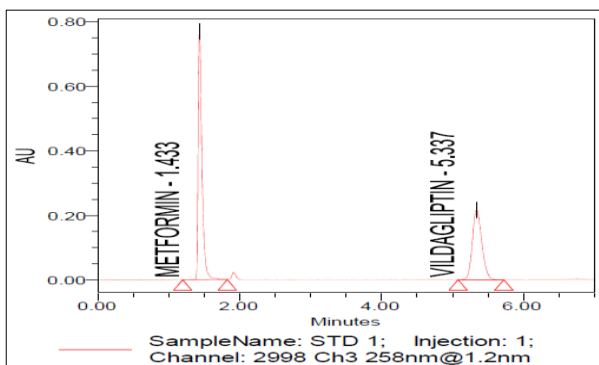
The optimization was performed by using the column Chromosil ODS C18 column 250 x 4.6mm 5μ, mobile phase 0.1M Di Potassium hydrogen phosphate and Methanol at the ratio (60:40%v/v) adjust the pH:9.2 by using Ortho phosphoric acid. The flow rate was 0.5ml/min and detection was observed at 258nm. The retention time of Metformin and Vildagliptin was 1.43min and 5.32min respectively.

**Table 2:** System suitability parameters of Galvusmet tablet

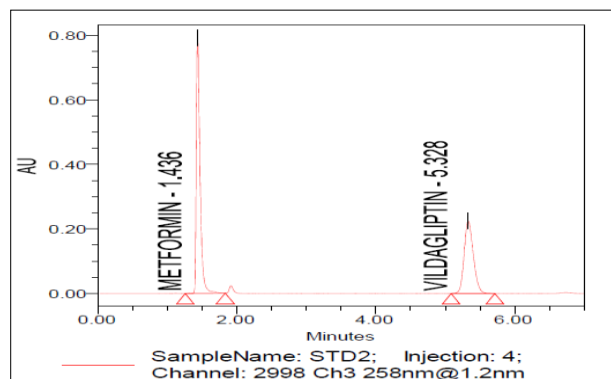
| S. No. | Parameter          | Vildagliptin | Metformin |
|--------|--------------------|--------------|-----------|
| 1.     | Retention Time     | 5.337        | 1.433     |
| 2.     | Tailing            | 1.181        | 1.721     |
| 3.     | Theoretical Plates | 7594         | 3621      |
| 4.     | %RSD(n=5)          | 0.2          | 0.4       |
| 5.     | Similarity Factor  | 0.9816       | 0.9796    |

**Table 3:** Assay for vildagliptin and metformin hydrochloride tablet

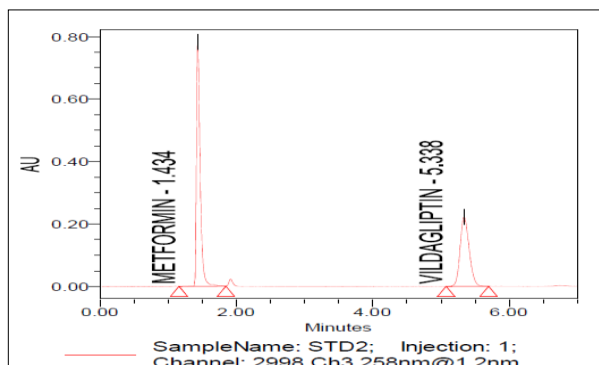
| S. No. | Name of the drugs | Mean sample peak area n=6 | Standard peak area | Labelled amount (Mg/Tab) | Amount found (Mg) | % Assay (95-105%) |
|--------|-------------------|---------------------------|--------------------|--------------------------|-------------------|-------------------|
| 1      | Vildagliptin      | 2085678                   | 2010811            | 50mg/ tablet             | 48.9mg            | 97.8              |
| 2      | Metformin         | 2893648                   | 2924916            | 500mg/ tablet            | 512mg             | 102.4             |



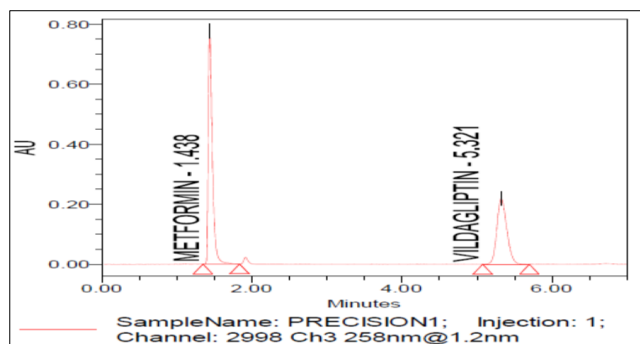
**Fig 1:** Typical chromatogram of mixed standard



**Fig 3:** Typical chromatogram of standard for specificity



**Fig 2:** Typical chromatogram of sample



**Fig 4:** Typical chromatogram of Precision

**Table 4:** Precision of metformin

| S.no.    | Sample     | Inj | Name      | RT    | Area    | USP tailing | USP plate count | S/N      |
|----------|------------|-----|-----------|-------|---------|-------------|-----------------|----------|
| 1        | PRECISION1 | 1   | METFORMIN | 1.438 | 2961791 | 1.731       | 3618            | 1567.542 |
| 2        | PRECISION2 | 1   | METFORMIN | 1.440 | 2995569 | 1.728       | 3576            | 1814.325 |
| 3        | PRECISION3 | 1   | METFORMIN | 1.439 | 2995425 | 1.739       | 3587            | 1685.032 |
| 4        | PRECISION4 | 1   | METFORMIN | 1.441 | 2999213 | 1.730       | 3588            | 1657.370 |
| 5        | PRECISION5 | 1   | METFORMIN | 1.441 | 2972181 | 1.694       | 3579            | 1953.594 |
| 6        | PRECISION6 | 1   | METFORMIN | 1.441 | 2989081 | 1.705       | 3615            | 1472.850 |
| Mean     |            |     |           | 1.44  | 2985543 | 1.721       | 3593            | 1691.785 |
| Std. Dev |            |     |           |       | 15102   |             |                 |          |
| %RSD     |            |     |           |       | 0.5     |             |                 |          |

**Table 5:** Precision of Vildagliptin

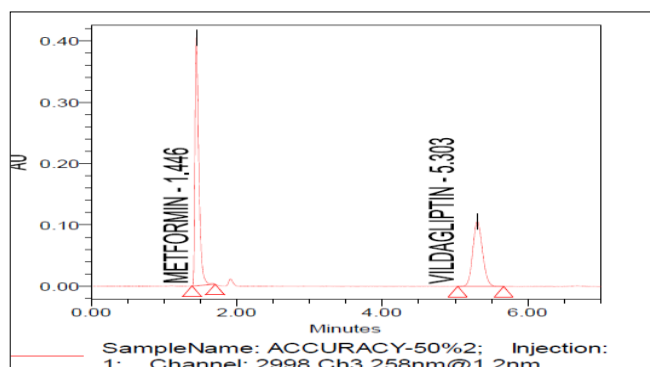
| S. No.   | Sample     | Inj | Name         | RT    | Area    | USP resolution | USP tailing | USP plate count | S/N     |
|----------|------------|-----|--------------|-------|---------|----------------|-------------|-----------------|---------|
| 1        | Precision1 | 1   | Vildagliptin | 5.321 | 2014022 | 22.451         | 1.189       | 7718            | 442.282 |
| 2        | Precision2 | 1   | Vildagliptin | 5.321 | 2032453 | 22.405         | 1.186       | 7649            | 510.228 |
| 3        | Precision3 | 1   | Vildagliptin | 5.314 | 2026948 | 22.280         | 1.193       | 7522            | 471.588 |
| 4        | Precision4 | 1   | Vildagliptin | 5.313 | 2034497 | 22.391         | 1.185       | 7650            | 464.966 |
| 5        | Precision5 | 1   | Vildagliptin | 5.312 | 2059452 | 22.460         | 1.187       | 7821            | 556.683 |
| 6        | Precision6 | 1   | Vildagliptin | 5.307 | 2053236 | 22.534         | 1.191       | 7837            | 419.727 |
| Mean     |            |     |              | 5.314 | 2036768 | 22.420         | 1.188       | 7699            | 477.579 |
| Std. Dev |            |     |              |       | 16872   |                |             |                 |         |
| %RSD     |            |     |              |       | 0.8     |                |             |                 |         |

**Table 6:** Assay of precision

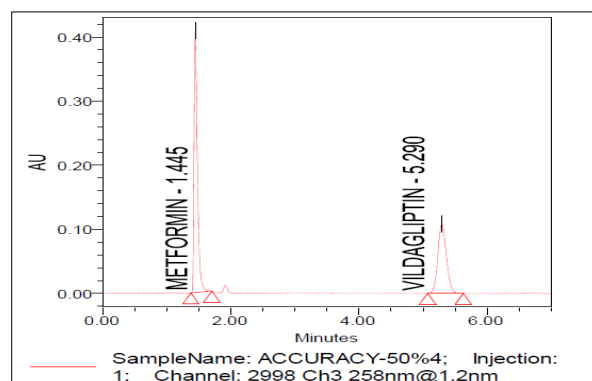
| S. No.         | Sample weight | Sample area of vildagliptin | Sample area of metformin | %Assay of vildagliptin | %Assay of metformin |
|----------------|---------------|-----------------------------|--------------------------|------------------------|---------------------|
| 1              | 1246          | 2014022                     | 2961791                  | 98                     | 99                  |
| 2              | 1246          | 2032453                     | 2995569                  | 99                     | 100                 |
| 3              | 1246          | 2026948                     | 2995425                  | 99                     | 100                 |
| 4              | 1246          | 2034497                     | 2999213                  | 99                     | 100                 |
| 5              | 1246          | 2059452                     | 2972181                  | 100                    | 99                  |
| 6              | 1246          | 2053236                     | 2989081                  | 100                    | 100                 |
| Std. deviation |               | 16872                       | 15102                    | 0.82                   | 0.51                |
| % RSD          |               | 0.8                         | 0.5                      | 0.83                   | 0.51                |

**Table 7:** Data of Intraday precision

| S. No.  | Sample Weight | Vildagliptin Area | Metformin Area | %Assay of Vildagliptin | %Assay of Metformin |
|---------|---------------|-------------------|----------------|------------------------|---------------------|
| 1       | 1246          | 2094158           | 2953216        | 102                    | 99                  |
| 2       | 1246          | 2034087           | 2997291        | 99                     | 100                 |
| 3       | 1246          | 2024507           | 2971913        | 99                     | 99                  |
| 4       | 1246          | 2026048           | 2973758        | 99                     | 100                 |
| 5       | 1246          | 2026917           | 2986560        | 99                     | 100                 |
| 6       | 1246          | 2020185           | 2983724        | 99                     | 100                 |
| Mean    |               | 2037650           | 2977744        | 99                     | 100                 |
| Std.Dev |               | 28047             | 15146          | 1.37                   | 0.51                |
| %RSD    |               | 1.4               | 1.1            | 1.36                   | 0.51                |



**Fig 5:** Typical chromatogram of accuracy 2 50%



**Fig 6:** Typical chromatogram of accuracy 4 50%

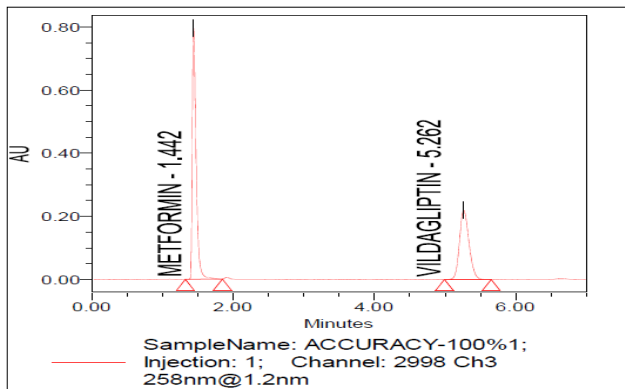


Fig 7: Typical chromatogram of accuracy 1 100%

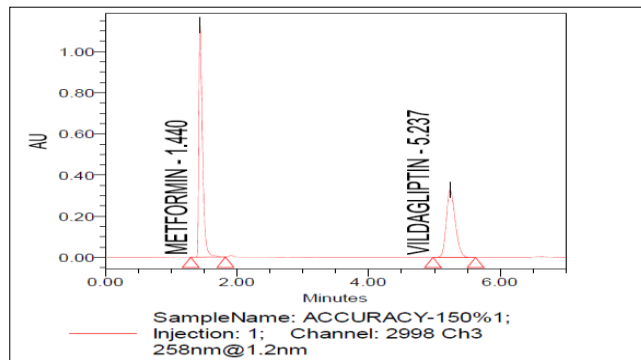


Fig 9: Typical chromatogram of accuracy 150%

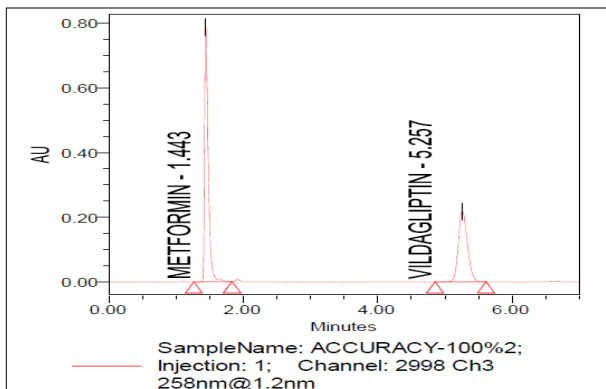


Fig 8: Typical chromatogram of ACCURACY 2 100%

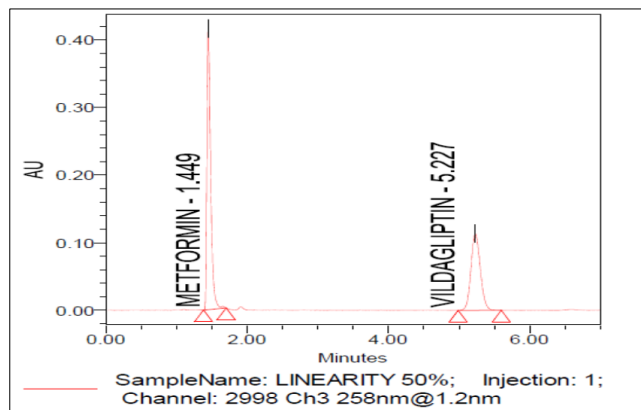


Fig 10: Typical chromatogram of linearity

Table 8: Accuracy of vildagliptin

| Spiked level | Sample weight | Sample Area | µg/ml added | µg/ml found | %Recovery | %Mean |
|--------------|---------------|-------------|-------------|-------------|-----------|-------|
| 50%          | 621.00        | 997834      | 199.358     | 194.62      | 98        |       |
| 50%          | 622.00        | 992244      | 199.679     | 193.53      | 97        |       |
| 50%          | 615.00        | 987707      | 197.432     | 192.64      | 98        | 97    |
| 50%          | 619.00        | 996135      | 198.716     | 194.29      | 98        |       |
| 50%          | 623.00        | 991543      | 200.000     | 193.39      | 97        |       |
| 50%          | 620.00        | 996664      | 199.037     | 194.39      | 98        |       |
| 100%         | 1246.00       | 2041682     | 400.000     | 398.21      | 100       |       |
| 100%         | 1245.00       | 2034527     | 399.679     | 396.81      | 99        | 100   |
| 100%         | 1246.00       | 2064285     | 400.000     | 402.62      | 101       |       |
| 150%         | 1869.00       | 3096685     | 600.000     | 603.97      | 101       |       |
| 150%         | 1868.00       | 3098866     | 599.679     | 604.40      | 101       |       |
| 150%         | 1869.00       | 3094341     | 600.000     | 603.52      | 101       | 101   |
| 150%         | 1867.00       | 3099493     | 599.358     | 604.52      | 101       |       |
| 150%         | 1869.00       | 3073826     | 600.000     | 599.52      | 100       |       |
| 150%         | 1869.00       | 3094182     | 600.000     | 603.49      | 101       |       |

Table 9: Accuracy of metformin

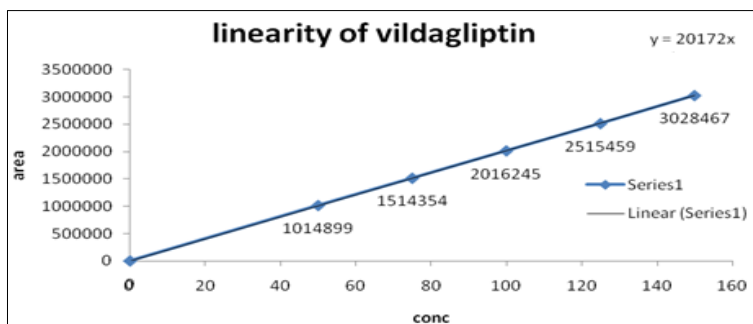
| Spiked level | Sample weight | Sample Area | µg/ml added | µg/ml found | %Recovery | %Mean |
|--------------|---------------|-------------|-------------|-------------|-----------|-------|
| 50%          | 621.00        | 1504436     | 1993.579    | 2014.35     | 101       |       |
| 50%          | 622.00        | 1508720     | 1996.709    | 2020.09     | 101       |       |
| 50%          | 615.00        | 1508932     | 1974.318    | 2020.37     | 102       | 101   |
| 50%          | 619.00        | 1500663     | 1987.159    | 2009.30     | 101       |       |
| 50%          | 623.00        | 1503577     | 2000.000    | 2013.20     | 101       |       |
| 50%          | 620.00        | 1503188     | 1990.369    | 2012.68     | 101       |       |
| 100%         | 1246.00       | 2998202     | 4000.000    | 4014.42     | 100       |       |
| 100%         | 1245.00       | 2980187     | 3996.709    | 3990.30     | 100       | 100   |
| 100%         | 1246.00       | 2989292     | 4000.000    | 4002.49     | 100       |       |
| 150%         | 1869.00       | 4426663     | 6000.000    | 5927.04     | 99        |       |
| 150%         | 1868.00       | 4414119     | 5996.790    | 5910.25     | 99        |       |
| 150%         | 1869.00       | 4430292     | 6000.000    | 5931.90     | 99        | 99    |
| 150%         | 1867.00       | 4425319     | 5993.579    | 5925.24     | 99        |       |
| 150%         | 1869.00       | 4434462     | 6000.000    | 5937.49     | 99        |       |
| 150%         | 1869.00       | 4454596     | 6000.000    | 5966.44     | 99        |       |

**Table 10:** Recovery of galvusmet

| S. No. | Recovery | Vildagliptin | Metformin |
|--------|----------|--------------|-----------|
| 1.     | 50%      | 97           | 101       |
| 2.     | 100%     | 100          | 100       |
| 3.     | 150%     | 101          | 99        |

**Table 11:** Linearity data of Metformin

| S.no. | Sample         | Inj | Name      | RT    | Area    | USP Tailing | USP Plate count | S/N      |
|-------|----------------|-----|-----------|-------|---------|-------------|-----------------|----------|
| 1     | Linearity 50%  | 1   | Metformin | 1.449 | 1486807 | 1.601       | 3864            | 769.974  |
| 2     | Linearity 75%  | 1   | Metformin | 1.446 | 2223941 | 1.625       | 3753            | 1254.816 |
| 3     | Linearity 100% | 1   | Metformin | 1.445 | 2963845 | 1.671       | 3780            | 1827.286 |
| 4     | Linearity 125% | 1   | Metformin | 1.446 | 3709470 | 1.764       | 3508            | 1829.243 |
| 5     | Linearity 150% | 1   | Metformin | 1.443 | 4446840 | 1.775       | 3416            | 2319.757 |



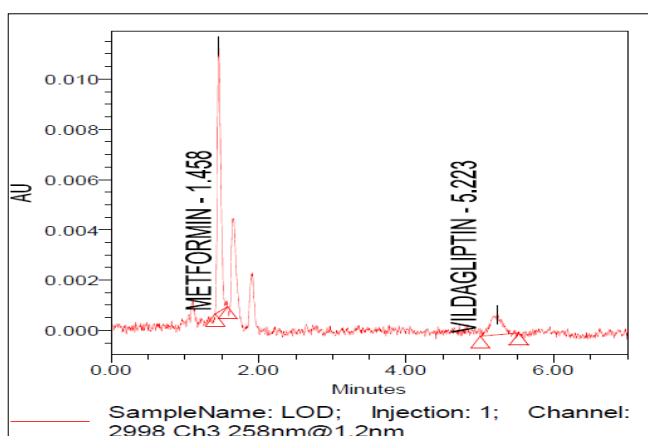
**Fig 11:** Linearity of Vildagliptin

**Table 12:** Linearity data of vildagliptin

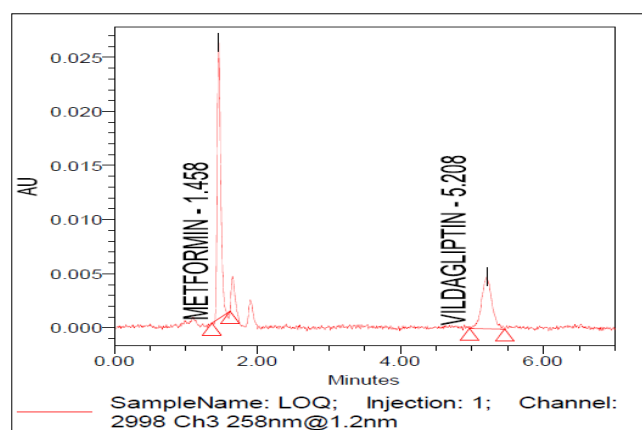
| S. No. | Sample         | Inj | Name         | RT    | Area    | USP Resolution | USP Tailing | USP Plate count | S/N     |
|--------|----------------|-----|--------------|-------|---------|----------------|-------------|-----------------|---------|
| 1      | Linearity 50%  | 1   | Vildagliptin | 5.227 | 1014899 | 22.203         | 1.102       | 7599            | 209.492 |
| 2      | Linearity 75%  | 1   | Vildagliptin | 5.212 | 1514354 | 22.418         | 1.095       | 7873            | 353.292 |
| 3      | Linearity 100% | 1   | Vildagliptin | 5.197 | 2016245 | 22.154         | 1.155       | 7643            | 512.435 |
| 4      | Linearity 125% | 1   | Vildagliptin | 5.192 | 2515459 | 21.390         | 1.188       | 7130            | 524.074 |
| 5      | Linearity 150% | 1   | Vildagliptin | 5.179 | 3028467 | 21.404         | 1.242       | 7178            | 683.893 |

**Table 13:** LOD&LOQ values of Galvusmet

| S. No. | Parameter | Vildagliptin | Metformin |
|--------|-----------|--------------|-----------|
| 1.     | LOD       | 0.0015       | 0.005     |
| 2.     | LOQ       | 0.0043       | 0.014     |



**Fig 12:** Typical Chromatogram of LOD



**Fig 13:** Typical Chromatogram of LOQ

**Table 14:** Comparison data of Robustness of Vildagliptin

| S. No. | Analyte     | Parameter          | Flow 0.4ml | Flow 0.6ml | Temp 33°C | Temp 237°C |
|--------|-------------|--------------------|------------|------------|-----------|------------|
| 1.     | Vildgliptin | Retention Time     | 6.392      | 4.367      | 5.236     | 5.160      |
| 2.     | Vildgliptin | Tailing            | 1.203      | 1.164      | 1.186     | 1.152      |
| 3.     | Vildgliptin | Theoretical Plates | 8465       | 7068       | 7752      | 7958       |

**Table 15:** Comparison data of Robustness of Metformin

| S. No. | Analyte   | Parameter          | Flow 0.4ml | Flow 0.6ml | Temp 33°C | Temp 37°C |
|--------|-----------|--------------------|------------|------------|-----------|-----------|
| 1.     | Metformin | Retention Time     | 1.805      | 1.209      | 1.450     | 1.447     |
| 2.     | Metformin | Tailing            | 1.748      | 1.749      | 1.749     | 1.710     |
| 3.     | Metformin | Theoretical Plates | 3576       | 3540       | 3584      | 3615      |

**Table 16:** Forced degradation data of Galvusmet

| S.no.    | Sample weight | Vildagliptin Area | Metformin Area | %Assay of vildagliptin | %Assay of metformin | %Degradation of vildagliptin | %Degradation Of metformin |
|----------|---------------|-------------------|----------------|------------------------|---------------------|------------------------------|---------------------------|
| Heat     | 1246          | 1894179           | 2745878        | 94                     | 94                  | -6                           | -6                        |
| Acid     | 1246          | 1563464           | 2054657        | 78                     | 70                  | -22                          | -30                       |
| Base     | 1246          | 1616193           | 2014049        | 80                     | 69                  | -20                          | -31                       |
| Peroxide | 1246          | 1519582           | 1836252        | 76                     | 63                  | -24                          | -37                       |
| Light    | 1246          | 1871891           | 2782466        | 93                     | 95                  | -7                           | -5                        |

#### 4. Conclusion

The estimation of Metformin hydrochloride and Vildagliptin by simultaneous method using RPHPLC method was performed by using Chromosil ODS C<sub>18</sub> column having 250 x 4.6mm 5 $\mu$ , and mobile phase containing 0.1M Di Potassium hydrogen phosphate and Methanol at the ratio (60:40%v/v) adjust the pH:9.2 by using Ortho phosphoric acid. The flow rate was 0.5ml/min and detection was observed at 258nm. The retention time of Metformin and Vildagliptin was 1.43min and 5.32min respectively. The all validation parameters were accepted within the limits as per ICH guidelines. The specificity was performed under stress conditions like acid, base, peroxide, light and heat and observed the degradation of drugs. It was found to be within the acceptance limit. Hence it was concluded that the developed method was found to be simple, precise, accurate and rapid method for the routine analysis of Vildagliptin and Metformin hydrochloride API and pharmaceutical dosage form in laboratories.

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