Development and Validation of Novel Ultra Performance Liquid Chromatography Technique for the Simultaneous Determination of Metformin and Repaglinide in Bulk and Formulation

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Authors’ contributions
This work was carried out in collaboration among all authors. Author HKSK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SA and Author PKP managed the analyses of the study. Author PKP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A novel ultra-performance liquid chromatographic technique for the estimation of metformin and repaglinide in an API and tablet dosage form. The chromatographic separation was achieved using DIKMA Endoversil (2.1 x 50mm, 1.7µm) column with a mobile phase of phosphate buffer, pH 4.2 and methanol as a mobile phase (38:62) with a flow rate of 0.3 mL/min and the detection wavelength was monitored at 241 nm. The method was validated in accordance with International conference on harmonization guidelines. In this present method metformin was eluted at 0.516 minute and repaglinide was eluted at 1.152 min. Limit of detection was 0.05 µg/ml for metformin and 1.152 µg/ml for repaglinide limit of quantification was found 0.5 µg/mL. Calibration curve plots were found linear over the concentration ranges 1-50 µg/mL for both the analytes. The % assay of...
the marketed dosage form was found 99.45 % for metformin and 97.08 % for repaglinide. The present study approach was found to be effective in the analysis of both analytes in force degradation conditions, because both the analytes has been specifically eluted in presence of other chromatograms. The experiential evidences of all the study results revealed the suitability of the estimation of metformin and repaglinide in API and tablet dosage form.

Keywords: Metformin; repaglinide; UPLC; method development; ICH Guidelines.

1. INTRODUCTION

Metformin hydrochloride; (1-carbamimidamido-N, N dimethyl methan imidamide is an orally administered antihyperglycemic. It improves control of glycemia primarily by inhibiting hepatic gluconeogenesis and glucogenolysis [1]. Repaglinide (Rep); 2-ethoxy-4-[2-(3-methyl-1-[2-(1-piperidinyl) phenyl] butylamino)-2-oxoethyl] benzoic acid (Fig. 1) is a fast acting prandial oral hypoglycemic agent for patients with non-insulin-dependent diabetes mellitus [1]. It reduces the fasting glucose concentrations by increasing the amount of insulin released by the pancreas [2]. This particular diabetes medication is a combination of 2 drugs (repaglinide and metformin). It is used along with a diet and exercise program to control high blood sugar in patients with type 2 diabetes. Repaglinide works by stimulating the release of body’s natural insulin. Metformin is a biguanide and works by decreasing the amount of sugar that your liver makes and that our stomach/intestines absorb. Both of these medications work by helping to restore your body’s proper response to the insulin you naturally produce [3,4]. Repaglinide/metformin fixed-dose combination (FDC) therapy (PrandiMet®, Novo Nordisk, Bagsvaerd, Denmark) has been approved for use in the USA. This FDC is a rational second-line therapy given the complementary mechanisms of action of the components [5]. The extensive review of literature on the estimation of present combination of repaglinide and metformin reveals that few RP-HPLC methods was reported including one HPTLC method [6]. The literature review also revealed that this two analytes combination is not also mentioned in any pharmacopoeia till now. There was several HPLC methods were reported for the estimation of metformin with other antidiabetics [7-9]. Similarly one HPLC method [10], HPTLC method [11], and bioanalytical method [12] was established with repaglinide alone and with other antidiabetics. The reported HPLC methods for the present combination of repaglinide and metformin were found several pitfalls, which should bring into notice to establish the necessity of the novel method for this combination as explained below. The one reported method developed by Fauad et al. [13], where they presented ambiguous chromatogram and inconsiderable peak shape. There may be a problem of capacity factor. The linearity level was narrow (5-40 µg/mL). The retention time for repaglinide was 14.2 minutes, which is considered too long analysis time. In another reported method developed by prabhakar et al. [14], does not represent any HPLC chromatogram which evokes vagueness of the method. Love et al. [15] reported 6.13 minutes retention time for repaglinide, was considered as delayed analysis time. Raja et al. [16] reported quantitation limit of 6.307 µg/mL, which represents moderate sensitivity of the reported method. Patan et al. [17] developed the HPLC method for repaglinide and metformin and in abstract, concluded with the utilization of the same method for Zolpidem tartarate. This raised a question about how a developed method for repaglinide and metformin can be used for Zolpidem tartarate? This raised a huge ambiguity about specificity and reliability of the developed method. As a whole there is a several disadvantages on the previously reported developed method for this combination was noticed. After considering all the facts in the mind the authors understand the importance to have an ideal novel method for this combination. Therefore effort was taken to developed a fast reliable and validated method for this combination using ultra performance liquid chromatography (UPLC) which is considered more advanced, high-throughput and sensitive in comparison to HPLC [18].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Pharmaceutical grade working standards of metformin (99.97%) was obtained as a gift sample from Aurobindo pharmaceuticals, Shadnagar, Telangana and Repaglinide
(99.75%) was obtained from Dr. Reddy's Laboratories, Hyderabad, Telangana, India. The tablets of combined dosage form of Metformin and repaglinide were procured from the local market of Hyderabad. All chemicals and reagents were required for the method development and validation and stability Studies were purchased from Finer Limited, Fisher Scientific and Merck.

2.2 Instrumentation Conditions

The analysis was carried out using ultra performance liquid chromatography (UPLC) Acquity Waters, PDA detector with Empower 2 software equipped with auto Sampler. Detection was carried out at 241 nm using PDA detector. The utilised analytical balance was 0.1mg sensitivity (Afcoset ER-200A) and pH meter (Adwa – AD 1020). The DIKMA Endoversil (2.1 x 50mm, 1.7µm) UPLC Column with the flow rate 0.3ml/min (isocratic) was utilised.

2.3 Preparation of 0.05 M Phosphate Buffer

Accurately weighted 6.8 gram potassium dihydrogen orthophosphate and transferred into a 1000 ml flask, dissolved the entire content with 200 mL of distilled water and further diluted to 1000ml with HPLC water. The pH was adjusted to 4.2 with orthophosphoric acid.

2.4 Preparation of Mobile Phase

Accurately measured 380 ml (38 %) of prepared 0.05 M phosphate buffer and 620 ml of HPLC grade methanol (62 %) were mixed well and degassed using an ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

2.5 Preparation of Standard Stock Solution

Accurately transfer 100 mg of metformin and 20mg of repaglinide in a volumetric flask. 10 mL of HPLC grade methanol was added to dissolve the content and further the volume was made up to 100 mL using prepared buffer solution to achieve 1 mg/mL of metformin and 0.2 mg/mL of repaglinide.

2.6 Preparation of Sample Solution

Accurately transferred 1 mL of aliquot from the standard stock solution and diluted to 100 mL using mobile phase. The final concentrations of 10 µg/mL of metformin and 2 µg/mL of repaglinide was achieved.

2.7 Sample Preparation for the Assay of Marketed Dosage Form

Accurately weigh 10 “Prandimet” tablets (manufactured and marketed by Lupin Pharmaceuticals), and triturated in a mortar and pestle and transfer the tablet powder equivalent to 1000 mg metformin and 4 mg of repaglinide into a 100 ml clean dry volumetric flask add about 10 ml of methanol and sonicate it up to 5 mins to dissolve completely and make volume up to the mark using mobile phase. Then it was filtered through 0.44 micron injection filter. From the above solution accurately 0.5 mL was withdrawn and transferred to a 10ml volumetric flask and volume was made up to the mark with mobile phase to achieve 500µg/mL of metformin and 2 µg/mL of repaglinide. Further 2 mL aliquot was withdrawn and transferred to 10 mL volumetric flask to volume was made to achieve the final diluted tablet solution which contains 100 µg/mL of metformin and 0.4 µg/mL of repaglinide. This final solution was injected into the UPLC system and areas was measured for both the analytes. Finally the percentage assay was calculated in triplicate.

2.8 Method Validation

2.8.1 System suitability

This research was conducted [19] to determine whether the analytical system is functioning properly. It has been carried out by injecting six replicates of metformin and repaglinide combined working standard solution. The percent RSD of various optimized parameters, such as peak area, retention time, and asymmetric factor, was calculated.

2.8.2 Specificity

It was done using a placebo intervention test of the sample solution, which involved dissolving 500 mg of placebo (equivalent to one tablet) in 100ml of mobile phase and treating the placebo solution as a normal solution. The solution was injected into the chromatographic device to see if any interfering peaks were present.

2.8.3 Accuracy

The recovery study was performed using the combined metformin and repaglinide solution at
different levels to justify the accuracy of the developed procedure (80 percent, 100 percent, and 120 percent). To achieve the various levels, different quantities of standard metformin and repaglinide were added to the combined tablet sample solution at a fixed concentration. This study [20] was repeated three times, with the percentage recovery and percentage mean recovery measured each time.

2.8.4 Intra day & Inter day precision

By determining the prepared standard solution of metformin and repaglinide, the precision of this developed method was investigated [21]. It was carried out by analysing six sample solutions containing 10 µg/mL metformin and 2 µg/mL repaglinide in triplicate. The intra- and inter-day precision was calculated by analysing six times on the same day (intra-day study) and repeating the process six times more on the following six days (inter-day study). The chromatograms from the UPLC were reported. Both analytes’ peak area and retention time were determined.

2.8.5 Detection and Quantitation limit

The limit of detection was define as the concentration for which a signal-to-noise ratio of 3 was obtained and for Quantitation limit, a signal-to-noise ratio of 10 was considered [22]. Standard solutions of metformin and repaglinide was prepared as described earlier. From the working standard solution (10µg/mL of metformin + 2 µg/mL repaglinide) 0.5 mL was withdrawn and transferred in to the 10 mL of volumetric flask and volume was made up to the mark to obtained 0.5 and 0.1 µg/mL of metformin and repaglinide. This solution is used for the study of limit of quantitation. For the study of LOD, the further 1 mL aliquote was withdrawn from the above sample and filled up to the mark to achieve the final concentration of 0.05 and 0.01 µg/mL of metformin and repaglinide.

2.8.6 Linearity

Working standard solutions of both analytes were prepared as described earlier, and aliquots from these solutions were diluted with mobile phase in five different concentrations to yield linearity solutions ranging from 1 to 50 µg/mL. The obtained data was subjected to regression analysis after a calibration curve was plotted for both the drug under study and the concentration versus peak area.

2.8.7 Robustness

By systematically adjusting the chromatographic conditions, the robustness of the established method was investigated [22]. Six sample solutions of metformin and repaglinide were prepared and analysed in triplicate under optimised conditions, with analytical conditions such as flow rate, mobile phase ratio, and detection wavelength varied at three different levels. Robustness study was performed by changing in the flow rate from 0.2, 0.3, 0.4 ml/min, buffer and methanol ratio from (60: 40, 62: 38 and 64: 36), detection wavelength of 240, 241 and 242 nm. The tailing factor was taken into account as a parameter and percentage RSD was calculated

![Chemical structure of Metformin (A) and Ripaglinide (B)](image)

Fig. 1: Chemical structure of Metformin (A) and Ripaglinide (B)
2.9 Force Degradation Study of Darunavir

The force degradation study [23] was carried out under various ICH-recommended stress conditions, including acidic, basic, oxidative, thermal, and photolytic stress. All forms of degradation studies were carried out in triplicate, with the mean peak area factored into the calculations.

2.9.1 Acid degradation

The analytes’ acid degradation was studied using 1M HCL in an environmental test chamber (Acamus Technologies, India) at 60°C and 75% relative humidity. In a 10 ml volumetric flask, 0.5 ml of stock solution (1 mg/mL) was applied, followed by 0.5 ml of 1 M HCL, and the flask was kept in an environmental test chamber for 16 hours. Following the stress cycle, the solution was neutralized with 1M NaOH and the volume was made up with mobile phase.

2.9.2 Base degradation

The metformin and repaglinide degradation studies were carried out in the same environmental chamber at 60°C and 58% relative humidity. In a 10 ml volumetric flask, 0.3 ml of stock solution was combined with 1 M 0.3 ml of 1M NaOH for 16 hours. The solution was neutralized with 1 M HCL and the volume was made up with mobile phase after the appropriate stress time.

2.9.3 Oxidative degradation

It was carried out in a flexible environmental chamber at 40°C, 75% relative humidity, and 6% H₂O₂. For this, 0.3 ml of stock solution was placed in a 10 ml volumetric flask, and 0.3 ml of 6% H₂O₂ was added to the flask, which was then held at 60°C for 16 hours before making up the volume with mobile phase.

2.9.4 Thermal degradation

It was carried out in an environmental chamber at 40°C with a relative humidity of 75%, in an oven at 105°C with 0.3 ml of stock solution in a 10 ml volumetric flask held in the chamber for 14 hours, and for dry heat thermolysis, 1 mg of dry drug in solid form was put in an oven at 110°C for 2 days.

2.9.5 Photolytic degradation

This experiment was lasted 48 hours and used UV light at a wavelength of 254 nm. For the experiment, 0.3 mL of stock solution was placed in a 10 mL volumetric flask and filled to the desired volume with mobile phase.

3. RESULTS AND DISCUSSION

Using an RP 18 Endoversil (2.1cm x 50 mm, 1.7m) column, the selected mobile phase of phosphate buffer and methanol in a volume ratio of 62: 38 (v/v) was adjusted to 4.2 with acetic acid, with the apparent pH of the mixture being adjusted to 4.2. Metformin and repaglinide had retention times of 0.516 and 1.152 minutes, respectively. The ideal working temperature was discovered to be 30°C Celsius. As shown in Fig. 2, a 10 µl injection volume with PDA detection at 241 nm was found to be optimal. For the following study, the above condition was used.

Fig. 2. Optimized UPLC chromatogram of Metformin and ripaglinide
3.1 Methods Validation

The method's accuracy was tested by determining the percentage of spiked crude drugs recovered at three different levels: 80%, 100%, and 120%. The accuracy of the method is shown in Table 1 with a mean recovery (n=6) of 98.59% for metformin and 99.22% for repaglinide. Metformin and repaglinide have percent RSD values of 0.39 and 0.52, respectively, according to intra-day precision analysis (n=6). As shown in Table 1, the mean content of the inter-day precision was 0.77 percent RSD for metformin and 0.43 percent RSD for repaglinide. The linearity of the established method was confirmed by the regression coefficient $R^2$ of 0.997 for metformin and 0.999 for repaglinide. The results of the system suitability study using standard metformin and repaglinide solution in six replicated exhibited that the % RSD of several parameters (theoretical plate, retention time, peak area, and asymmetric factor) were within the acceptable range, i.e. less than 2, as shown in the table, indicating the analytical system’s suitability to perform the process. At the retention time corresponding to both metformin and repaglinide, no peak was observed in the analysis of specificity. The LOD value for metformin was 0.05 g/mL and for repaglinide was 0.01 g/mL, according to the normal signal to noise ratio formula. Metformin has a LOQ of 0.5 g/mL, while repaglinide has a LOQ of 0.1 g/mL. The mean percent RSD of the retention time and peak area in all adjusted conditions was found to be 1.37 for metformin and 0.82 for repaglinide in the analysis of robustness, which is within acceptable limits. The percent assay for metformin was 99.45 percent and 97.08 percent for repaglinide in the fixed dose combination tablet “PRANDIMET,” which contains 500 mg of metformin and 2 mg of repaglinide. The outcome is shown in Table 1. Fig. 3 depicts the UPLC chromatogram of a commercial formulation.

Table 1. Summary of validation parameters

| Parameters                                      | Metformin | Repaglinide |
|------------------------------------------------|-----------|-------------|
| Linearity range ($\mu$g/ml)                    | 1-50      | 1-50        |
| Co-relation co-efficient                        | 0.997     | 0.999       |
| LOD $\mu$g/ml                                  | 0.15      | 0.25        |
| LOQ $\mu$g/ml                                  | 0.5       | 0.75        |
| Repeatability (% RSD)                          | 0.39      | 0.52        |
| Intermediate precision (% RSD)                 | 0.77      | 0.43        |
| Mean % recovery                                | 98.59     | 99.22       |
| % Assay (Prandimet Tablets, each tablet contains 500mg Metformin + 2 mg of Repaglinide) | 99.45     | 97.08       |
| %RSD of Tailing factor* (Robustness study)     | 1.37      | 0.82        |

*average of all deliberately modified conditions

![UPLC chromatogram of Metformin and repaglinide tablet dosage form](image)
The Force degradation analysis was carried out under optimised conditions, and the stressed samples' assay values were determined. At alkaline tension, metformin degrades at a rate of 4.97%, while repaglinide degrades at a rate of 2.83%. Metformin degrades 3.19% in acidic stressed conditions, while repaglinide degrades 2.41%. Table 2 contains the specifics of the other findings. Fig. 4 shows UPLC chromatograms of degradation.

**Table 2. Degradation study results for metformin and repaglinide**

| Metformin       | Sample | Mean Area | % Degraded | Purity Angle | Purity Threshold | Peak purity |
|-----------------|--------|-----------|------------|--------------|------------------|-------------|
| Metformin       | Acid   | 499332    | 3.19       | 0.578        | 1.546            | Passes      |
|                 | Base   | 485917    | 4.97       | 0.792        | 2.364            | Passes      |
|                 | Peroxide | 509398   | 2.05       | 0.303        | 0.564            | Passes      |
|                 | Thermal | 500224   | 2.84       | 0.323        | 1.152            | Passes      |
|                 | Photo  | 516745    | 0.66       | 0.285        | 0.431            | Passes      |

| Repaglinide     | Acid   | 170982    | 2.41       | 0.765        | 1.442            | Passes      |
|                 | Base   | 168456    | 2.83       | 0.976        | 2.032            | Passes      |
|                 | Peroxide | 179450  | 1.27       | 0.342        | 1.234            | Passes      |
|                 | Thermal | 178456   | 1.02       | 0.231        | 0.723            | Passes      |
|                 | Photo  | 186540    | 0.72       | 0.187        | 0.437            | Passes      |

Fig. 4. UPLC chromatograms of Metformin and repaglinide force degradation samples
Based on the obtained results, the present developed method can be confer as follows. For the optimization and development of this method number of preliminary trials were performed with Various solvents (methanol, acetonitrile), as well as buffers (orthophosphoric acid pH 2, 8, ammonium acetate pH 5 and 7, potassium dihydrogen orthophosphate pH 5, 6.8), were used in various volume ratios. The pH, flow rate, temperature, and varieties of columns (C-8, C-18) has been utilised in order to justify the retention time, peak shape, resolution, and other chromatographic peak parameters before selecting the optimal chromatographic condition for the study of metformin and repaglinide. The mobile phase for the optimization procedure were made by combining different buffer systems with organic solvents.

The temperature of the column was ranged from 20° to 40° Celsius. The mobile phase (A) containing potassium dihydrogen phosphate buffer and methanol (B) (A: B 50:50 v/v) was also tested with the PDA detector at 223 nm. Finally, the apparent pH of the mixture was changed to 4.2 with acetic acid using the DIKMA Endoversil C-18 (2.1 x 50mm, 1.7m) UPLC column with a flow rate of 0.3ml/min (isocratic) and a selected mobile phase consisting of phosphate buffer and methanol in the volume ratio of 32:68(v/v). Metformin and repaglinide were completely isolated under these chromatographic conditions, with retention times of 0.516 and 1.152 minutes for metformin and repaglinide respectively, and better sensitivity and peak shape with acceptable resolution. The sensitivity of both the drugs under analysis and the PDA detector with a selected wavelength of 241 nm is greatly improved. The intra-day and inter-day precision values were presented as percent RSD, indicating that the established method was significantly accurate. In a particular range, a linear correlation was revealed between concentration and peak area. For the system suitability analysis of metformin and repaglinide, many parameters such as theoretical plate, retention time, peak area, and asymmetric factor were considered and found to be within limits. The results of the specificity analysis clearly correlate that no excipients interfere was found with the formulation and strongly support the specificity of the established process. The sensitivity of the established method is clearly indicated by the LOD and LOQ values. The robustness study revealed that with the deliberate changes in flow rate and temperature, there was little variance in tailing factor. The tailing factor was found to be within the study's parameters. Both metformin and repaglinide were found to be very similar to the labeled claim number, indicating that the assay method for determining drug content is very effective. The peaks of both analytes repaglinide and metformin were well isolated and found to be very specific from the degradation peaks depicted in the chromatograms, according to the force degradation report. The peak area of metformin and repaglinide was reduced in some stressed environments. Additional peaks due to analyte degradation in various conditions revealed that both the drugs were degraded to an extent in alkaline stressed conditions, marginally less in acidic stressed conditions, and to a lesser extent in oxidative stressed conditions. In the photolytically strained environments, there was very little to no degradation was observed. The chromatogram resembled a fresh sample before being stressed, indicating that both drugs were significantly stable under photolytic stress.

4. CONCLUSION

Based on the empirical evidences of this developed method, authors can be firmly assert about the novelty of the present developed method over the available methods. This is the first stability-indicating UPLC procedure for metformin and repaglinide, and it is referred to as "rapid" because the overall study time was greatly reduced. The current approach is called "stability suggesting" because it has shown a less pronounced degradation pattern in stressed conditions and strong distinction between the other degraded peaks. The results of all validation parameters met the ICH Q2B guidelines' acceptance requirements. Hence the present established method can be employed as a novel, accurate, validated useful method can be apply for routine analytical and quality control assay of metformin and repaglinide in the tablet dosage form.

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.
CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Neil JO. The Merck index, 13th ed., pharmaceutical Press, London, UK; 2007.
2. Hardman JG, Limbard LE. Goodman Gillman, "The pharmacological basis of therapeutics", 11th ed., Mc Graw Hill; 2005.
3. Rudenski AS, Hadden DR, Atkinson AB. Natural history of pancreatic islet B-cell function in type 2 diabetes mellitus studied over six years by homeostasis model assessment. Diabet Med. 1988;5:36–41.
4. Rodbard HW, Davidson JA, Garber AJ, Handelsman Y, Lebovitz H, Moghissi ES. Statement by an American Association of Clinical Endocrinologists/American College of Endocrinology consensus panel on type 2 diabetes mellitus: an algorithm for glycemic control. Endocr Pract. 2009;15:540–559.
5. Robert GM. Repaglinide/metformin fixed-dose combination to improve glycemic control in patients with type 2 diabetes: an update. Diabetes Metab Syndr Obes. 2010;3:145–154.
6. Ahir KB, Patelia EM, Shah A. Simultaneous Estimation of Metformin Hydrochloride and Repaglinide in Pharmaceutical Formulation by HPTLC-Densitometry Method. J Chromat Separation Techniq. 2013;4:166.
7. Sudhir Adsul Jayant Bidkar Sunil Harer GY. Dama. RP- HPLC Method Development and Validation for Simultaneous Estimation for Metformin and Sitagliptin in Bulk and Tablet Formulation, Int J of Chem Tech Res. 2018;11(11):428-435.
8. Elkady EF, El-Zaheer AA, Elwy MY, Saleh MA. Validated Liquid Chromatographic Method for Simultaneous Determination of Metformin, Pioglitazone, Sitagliptin, Repaglinide, Glimepiride and Gliclazide - Application for Counterfeit Drug Analysis. J Anal Bioanal Tech. 2015;S13:007.
9. Karimulla SK, Vasanth PM, Ramesh T2, Ramesh M. Method development and validation of sitagliptin and metformin using reverse phase HPLC method in bulk and tablet dosage form, Der Pharm Let. 2013;5(5):168-174.
10. Pingale PL. Nandasana PV. “Development and Validation of RP-HPLC Method for the Estimation of Repaglinide in Bulk Drug and Pharmaceutical Formulation” Int. J. Drug Dev. & Res.2012, 4(3): 247-252.
11. Kiran Deshpande, Pranjali Ranaware AR, Madgulkar MC. Damle. Development and Validation of Stability Indicating HPTLC Method for Determination of Repaglinide. Research J. Pharm. and Tech. 2013, 6(2): 158-161.
12. Ruzilawati AB, Wahab MSA, Ismail AI, Gan, SH. Method development and validation of repaglinide in human plasma by HPLC and its application in pharmacokinetic studies. J Pharm Biomed Anal. 2007;43(5):1831-1835.
13. Fouad M, Rashed NS. Development and validation of chromatographic and spectroscopic methods for estimation of repaglinide and metformin hcl in combined dosage form. J of global trends in pharm sci. 2014;5(3):1844-1848.
14. Prbhakar S, Harshini, D. Sireesha, M. Haque A, Bakshi, V. Development and validation of analytical method for simultaneous estimation of metformin hcl and repaglinide in combined pharmaceutical dosage form by rp- hplc. International Journal of Medicine and Nanotechnology. 2014;1(3):163-168.
15. Soni LK, Narsinghani T, Jain M. Development and validation of rp-hplc method for simultaneous estimation of metformin hydrochloride and repaglinide in tablet dosage form, Journal of Liq Chromatogr & Rel Tech. 2012;35(3):385-392.16
16. Raja MA, Santhoshi R, Banji D, Rao KNV, Kumar DS. RP- HPLC method development and validation for
simultaneous estimation of metformin and repaglinide in bulk and tablet dosage form, Asian J Res Pharm Sci Biotech. 2015;3(2):33 - 40.

17. Patan A, Basha SR, Ketha RK, Cherian BV, Muthukumar VA, Development and Validation of New RP- HPLC Method for the Simultaneous Estimation of Metformin Hydrochloride and Repaglinide in Pure and Pharmaceutical Formulations. Research J. Pharm. and Tech. 2021;14(3):1323-1328.

18. Gumustas M, Kurbanoglu S, Uslu B. UPLC versus HPLC on Drug Analysis: Advantageous, Applications and Their Validation Parameters. Chromatographia. 2013;76:1365–1427.

19. Mondal P, Mahender K, Padmaja B., A Novel UPLC-PDA Method for the Simultaneous Determination of Lamivudine, Zidovudine and Nevirapine in Bulk and Tablet Dosage Form. Analytical Chem Let. 2018;8(1):131-8.

20. Mondal P, Kola V, A New Stability Indicating Validated RP-HPLC Method for Simultaneous Estimation of Escitalopram and Clonazepam in Bulk and Tablet Dosage Form. Asian J Pharm Anal. 2019;9(4):1-8.

21. Mondal P, Shobha SR, Raparla R. Novel Stability Indicating Validated RP-HPLC Method for Simultaneous Quantification of Artemether and Lufenantrine in Bulk and Tablet. Current Pharmaceutical Analysis. 2014;10:271-278.

22. Mondal P, Santosh B, Shobha SR, Raparla R. A new validated simultaneous RP- HPLC method for estimation of escitalopram oxalate and etizolam in bulk and table dosage form. Der Pharm Chem. 2013;5(3):26-32.

23. Klick S, Muijselaar PG, Waterval J, Eichinger T, Korn C, Gerding TK, et al. Toward a generic approach for: Stress testing of drug substances and drug products. Pharmaceutica Tech. 2005; 29(2):48-66.

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