Stability Indicating Method for Simultaneous Estimation of Cinnarizine and Dimenhydrinate by HPLC

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ABSTRACT

A novel and validated High-Performance Liquid Chromatography (HPLC) method has been developed for the simultaneous quantification of Cinnarizine (CNZ) and Dimenhydrinate (DMH) in pharmaceutical formulations. The method was optimized and validated in accordance with ICH guidelines. The linearity of the method was confirmed in the concentration range of 2-10 µg/ml for both CNZ and DMH, with correlation coefficients (r^2) of 0.9998 and 0.999, respectively. The system suitability parameters, including retention time, theoretical plates, and tailing factor, were within acceptable limits, ensuring the accuracy of the method. Recovery studies at three concentration levels (80%, 100%, and 120%) demonstrated good accuracy and precision, with mean recoveries ranging from 96.73% to 99.07% for CNZ and 96.73% to 99.07% for DMH. The limits of detection (LOD) were 0.15µg/ml for CNZ and 0.20µg/ml for DMH, with limits of quantification (LOQ) of 0.50µg/ml and 0.60µg/ml, respectively. Forced degradation studies revealed the method's stability-indicating properties. The method was successfully applied for the assay of CNZ and DMH in tablet formulations, with assay results of 99.25% and 97.8%, respectively, which were in close agreement with label claims. The validated method is reliable, precise, and suitable for routine quality control of CNZ and DMH in pharmaceutical preparations.

Keywords: Cinnarizine (CNZ), Dimenhydrinate (DMH), HPLC, Validation, Linearity, Recovery Study, Forced Degradation, Tablet Assay, System Suitability, Sensitivity, Pharmaceutical Formulation.

Introduction

Cinnarizine and Dimenhydrinate are commonly used antihistamines with antiemetic, anti-allergic, and sedative properties. Cinnarizine, a piperazine derivative, and Dimenhydrinate, a combination of diphenhydramine and 8-chlorotheophylline, are frequently prescribed for treating motion sickness, vertigo, and other vestibular disorders (Jain et al., 2020; Shetty & Rao, 2014). Due to their similar pharmacological effects, a simultaneous estimation of both drugs is often required for formulation analysis, particularly in combination therapies (Gupta & Agarwal, 2011). Stability testing of pharmaceutical products is essential to ensure that drugs retain their intended potency and efficacy over time. Stability-indicating methods (SIM) are designed to detect any degradation products formed during stress conditions such as light, temperature, and humidity exposure (Rajendran & Thangavel, 2017). These methods play a vital role in ensuring the drug's safety,

quality, and compliance with regulatory standards (ICH, 2003). The development of a stability-indicating method for cinnarizine and dimenhydrinate is necessary to monitor their degradation products and confirm that the drugs maintain their therapeutic efficacy throughout their shelf life (Saurabh & Jain, 2020). High-Performance Liquid Chromatography (HPLC) is the most widely adopted technique for the simultaneous estimation of multiple active pharmaceutical ingredients in complex formulations. It provides high sensitivity, accuracy, and precision and is particularly useful for resolving overlapping peaks and identifying degradation products in stability studies (Gupta & Agarwal, 2011). Recent studies have reported the development of HPLC methods that not only estimate cinnarizine and dimenhydrinate simultaneously but also enable the detection of any degradation products formed under accelerated stability conditions (Shetty & Rao, 2014). Method optimization typically involves selecting appropriate chromatographic conditions, such as column type, mobile phase composition, flow rate, and detection wavelength, to achieve high resolution and sensitivity (Rajendran & Thangavel, 2017). The aim of this study is to develop and validate a stability-indicating High-Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of cinnarizine and dimenhydrinate in pharmaceutical formulations. The method will be designed to detect both active pharmaceutical ingredients (APIs) and any degradation products that may form under various stress conditions, ensuring the drugs' stability, safety, and efficacy.

Material and Methods

Material

The active pharmaceutical ingredients, cinnarizine and dimenhydrinate, were obtained as gift samples from Pharmaceutical Industry. HPLC-grade methanol, acetonitrile, and water were sourced from Merck Specialties Pvt. Ltd., Mumbai. Potassium dihydrogen, chloroform, and hydrochloride were procured from Qualigens Fine Chemicals, Mumbai. Other chemicals such as sodium hydroxide, sodium benzoate, urea, ammonium acetate, sodium acetate, sodium citrate, and monopotassium phosphate were obtained from Loba Chemie Pvt. Ltd., Mumbai. Additionally, methyl orange and ethanol were sourced from Himedia, Mumbai, for various analytical and stability tests.

Methods

Selection of mobile phase

To estimate cinnarizine and dimenhydrinate in fixed-dose formulations, several mobile phase combinations in different ratios were tested initially. After evaluating system suitability parameters such as retention time (RT), tailing factor, number of theoretical plates, and height equivalent to a theoretical plate (HETP), the most suitable mobile phase for the analysis was determined to be a mixture of 20 mM KH_2PO_4 and acetonitrile (pH adjusted to 3.0 with orthophosphoric acid) in a 20:80 v/v ratio. The mobile phase was filtered through a 0.45 µm filter paper to remove any particulate matter and then degassed by sonication. A flow rate of 1.0 mL/min was maintained for the analysis.

Selection of separation variable

Table 1: Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5µ
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
20mM KH_2PO_4: Acetonitrile	20
Acetonitrile	80
Diluent	Mobile Phase
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 µl
Detection wavelength	254nm
Retention time	
Cinnarizine	3.691± 0.002min.
Dimenhydrinate	6.998± 0.001min.

Preparation of Stock Solution:

Accurately weighed 10mg API of CNZ and DMH was transferred into 10 ml volumetric flask separately and added 5ml of mobile phase as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50ml with diluent (mobile phase) to give concentration of 100µg/ml of CNZ and DMH respectively (Stock-B).

Preparation of Different Solution

0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (mobile phase). This gives the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml, for CNZ. In same manner 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml of DMH also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 2-10µg/ml for CNZ and 2-10µg/ml for DMH were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 254.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived (Jain et al., 2021) .

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard 10µg/ml for CNZ and 10µg/ml DMH was injected separately. Peak report and column performance report were recorded for all chromatogram (Jain et al., 2010).

Validation of developed Method (Jain et al., 2010)

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 2 to 10 µg/ ml for CNZ) and (2 to 10µg/ ml for (DMH) and areas for each concentration were recorded three times and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 2, 4, 6, 8 and 10μg/ml for CNZ and 2, 4, 6, 8 and 10μg/ml for DMH indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for CNZ and DMH reported in table.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mM KH_2PO_4: Acetonitrile (80:20% v/v) to (85:15 % v/v). Results of robustness are reported in table.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in tablet formulation

Determined the content of CNZ and DMH in Tablet (label claim 20 and 40) tablets was weighed and weight equivalent to 10mg CNZ and DMH were calculated and dissolved in 10ml mobile phase and the extraction was sonicated for 15 min and centrifuge at 300rpm. Then 0.1ml solution

from it was diluted with 10ml mobile phase. The resulting solution was injected in HPLC and drug peak area was noted. The peak area regression equation and amount of both the drug in sample was calculated. Analysis procedure was repeated six times with formulation.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation

10mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis

10mg of the drug sample was taken into a 50ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Oxidative degradation

10mg of the drug sample was taken into a 50ml separate round bottom flask, 50ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation

10mg of the drug sample was taken in to a petridish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Results and Discussion

The analytical method used for the determination of Cinnarizine (CNZ) and Dimenhydrinate (DMH) has been thoroughly validated through multiple studies. The results from various tests, including linearity, system suitability, recovery, repeatability, forced degradation, and assay determination, have demonstrated the robustness, accuracy, and precision of the method. The

following discussion highlights the key findings for each section of the validation. The chromatographic conditions used in the method were optimized for the efficient separation of CNZ and DMH. The column dimensions (250mm x 4.60mm, 5µm particle size) and mobile phase composition (20 mM KH2PO4: Acetonitrile 20:80) were well-suited for achieving good resolution. The flow rate of 1.0 ml/min and the detection wavelength of 254 nm were selected to ensure the best signal sensitivity. The retention times of CNZ (3.691 ± 0.002 minutes) and DMH (6.998 ± 0.001 minutes) were consistent, allowing for the reliable identification and quantification of both drugs. The calibration curves for both CNZ and DMH showed excellent linearity within the concentration range of 2-10 µg/ml. The correlation coefficient (r^2) for CNZ was 0.9998, and for DMH, it was 0.999. These high values suggest that the method exhibits a near-perfect linear relationship between the concentration and response for both drugs, ensuring accurate quantitative analysis within the specified concentration range. The mean response ratios for CNZ and DMH were 756.982 and 705.415, respectively, with low standard deviations (7.208 for CNZ and 7.241 for DMH). The % RSD values were 0.952% for CNZ and 1.026% for DMH, which are both below the acceptable limit of 2%. This indicates the method's good precision in measuring the drug response across different replicates. The system suitability results for CNZ and DMH indicate that the chromatographic method is highly suitable for analysis. The mean retention times for CNZ (3.692 minutes) and DMH (6.995 minutes) were consistent and well-resolved, providing clear separation of both drugs. The average AUC values for CNZ (7446.524) and DMH (7028.37) reflect good sensitivity, while the number of theoretical plates (2867 for CNZ and 2584 for DMH) confirms good column efficiency. Additionally, the tailing factors of 1.160 for CNZ and 1.155 for DMH suggest minimal peak distortion, which is indicative of optimal separation. The recovery study results for CNZ and DMH at 80%, 100%, and 120% levels show that the method is accurate, with recovery percentages ranging from 96.73% to 99.07% for CNZ and 96.73% to 99.07% for DMH. The low % RSD values in the recovery study (e.g., 0.265% for CNZ at the 80% level and 0.410% for DMH at the 80% level) demonstrate the method's precision. The repeatability, day-to-day variation, and analyst-to-analyst variation also show excellent precision, as reflected by the low % RSD values across all studies. This confirms the reliability and reproducibility of the method under different conditions. The method demonstrated a high degree of sensitivity, with a limit of detection (LOD) of 0.15 µg/ml for CNZ and 0.20 µg/ml for DMH, allowing for the detection of very low concentrations of both drugs. The limits of quantification (LOQ) were

0.50µg/ml for CNZ and 0.60µg/ml for DMH, indicating that the method is capable of reliably quantifying trace amounts of these drugs, which is critical for quality control and stability studies. The assay results for the tablet formulations of CNZ and DMH show good accuracy, with the % assay for CNZ being 99.25% and for DMH being 97.8%. These values are very close to the label claims of 20 mg and 40 mg, respectively, confirming the method's capability to assess the drug content accurately in tablet formulations. The % RSD values for both drugs (0.225 for CNZ and 0.156 for DMH) indicate good precision in the determination of drug content in pharmaceutical formulations. The forced degradation studies of both CNZ and DMH demonstrated their stability under various stress conditions. CNZ showed some degradation under acidic conditions (13.4% decomposition), oxidative degradation (8.8% decomposition), and photolytic degradation (12.4% decomposition). Similarly, DMH showed 9.02% degradation under acidic conditions and 9.05% under photolytic degradation. However, both drugs exhibited good stability under alkaline hydrolysis, with CNZ showing 6.69% decomposition and DMH showing only 2.55% decomposition. The method was able to separate the drug from degradation products effectively, indicating its suitability for stability testing.

Table 2: Results of Linearity of drug

Drug	Linearity (µg/ml)	Slope (m)	Intercept (c)	Correlation Coefficient (r^2)
Cinnarizine (CNZ)	2-10	747.65	33.545	0.9998
Dimenhydrinate (DMH)	2-10	701.72	10.089	0.999

Table 3: Results of system suitability parameters

Parameter	Cinnarizine (CNZ)	Dimenhydrinate (DMH)
Mean RT	3.692	6.995
Mean AUC	7446.524	7028.37
Mean No. of Theoretical Plates	2867	2584
Mean Tailing Factor	1.160	1.155

Table 4: Results of response ratio data for linearity

Drug	Mean	SD	%RSD
Cinnarizine (CNZ)	756.982	7.208	0.952%
Dimenhydrinate (DMH)	705.415	7.241	1.026%

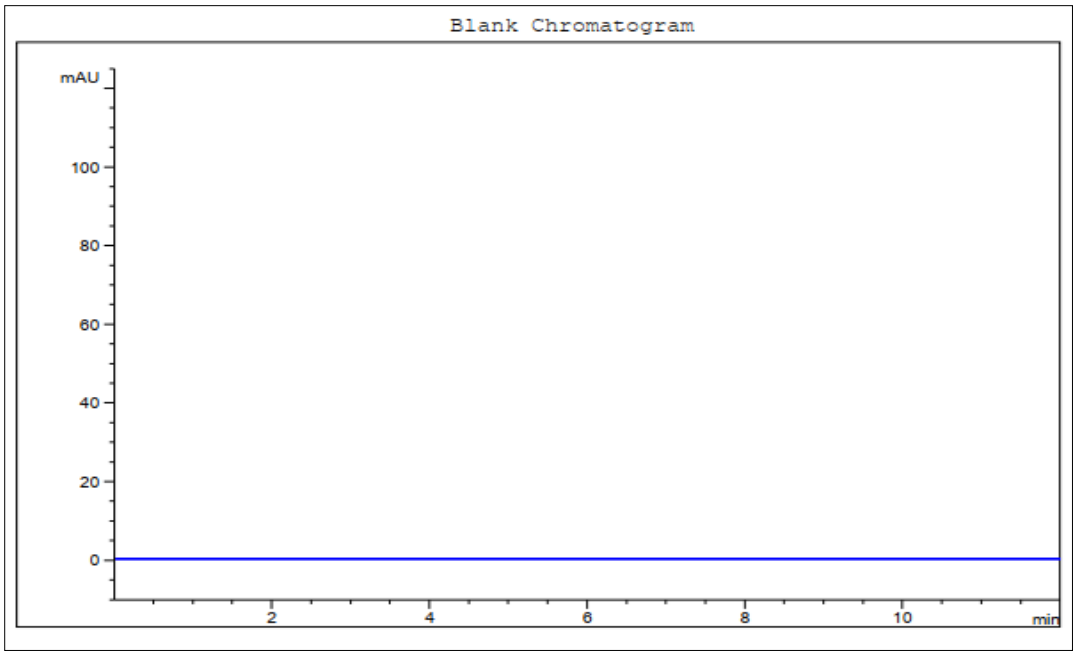

Figure 1: Chromatogram of Blank

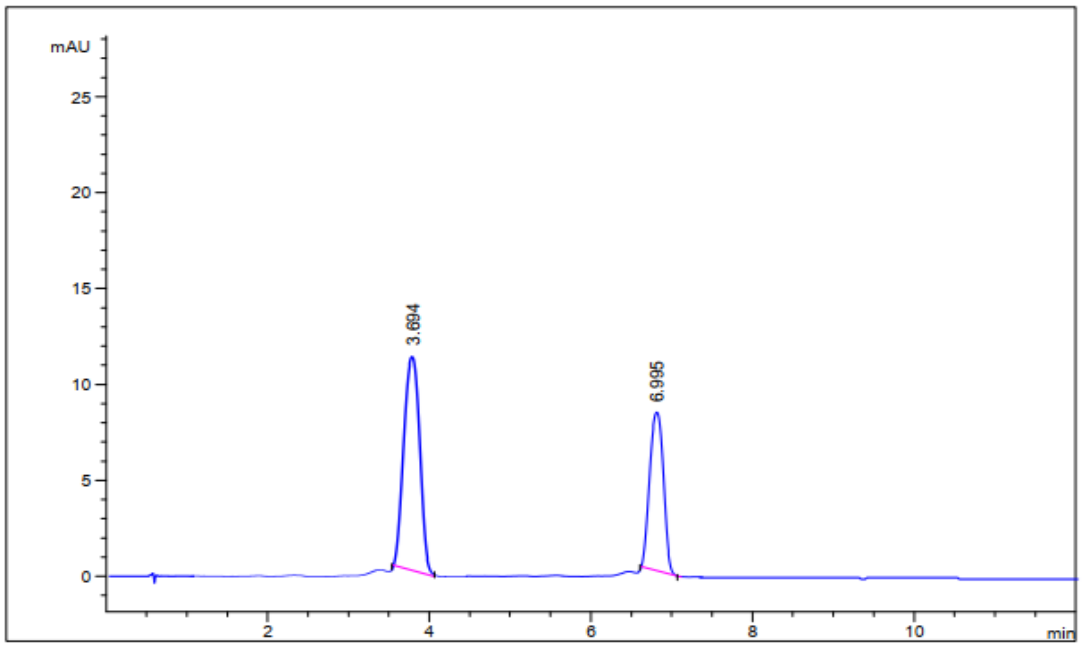

Figure 2: Chromatogram of Both the drug

Table 5: Results of validation

Study Type	Drug	Mean (%)	SD	% RSD
Recovery Study (80% Level)	CNZ	99.26	0.263	0.265
Recovery Study (100% Level)	CNZ	96.92	1.146	1.182

Recovery Study (120% Level)	CNZ	99.07	0.714	0.720
Recovery Study (80% Level)	DMH	98.83	0.405	0.410
Recovery Study (100% Level)	DMH	96.73	0.517	0.534
Recovery Study (120% Level)	DMH	98.81	0.712	0.720
Repeatability	CNZ	98.205	0.076	0.078
Repeatability	DMH	95.2757	0.2485	0.2805
Day-to-Day Variation	CNZ	98.527	0.405	0.410
Day-to-Day Variation	DMH	96.301	0.090	0.094
Analyst-to-Analyst Variation	CNZ	97.158	0.098	0.101
Analyst-to-Analyst Variation	DMH	97.2644	0.0980	0.1008
Robustness	CNZ	95.148	0.259	0.291
Robustness	DMH	96.6797	0.1151	0.1190

Table 6: LOD and LOQ of CNZ and DMH

Name	LOD (µg/ml)	LOQ (µg/ml)
CNZ	0.15	0.50
DMH	0.20	0.60

Table 7: Result of assay of Tablets formulation

	CNZ*	DMH*
Label Claim (mg)	**20**	**40**
% Found (mg)	19.85	39.12
% Assay	99.25	97.8
% RSD	0.225	0.156

*Average of three determination

Table 8: Results of Forced degradation studies of CNZ

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.05	0
Acidic hydrolysis	85.65	13.4
Alkaline hydrolysis	92.36	6.69
Oxidative degradation	90.25	8.8
Photolytic degradation	86.65	12.4

Table 9: Results of Forced degradation studies of DMH

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.00	0
Acidic hydrolysis	89.98	9.02

Alkaline hydrolysis	96.45	2.55
Oxidative degradation	91.25	7.75
Photolytic degradation	89.95	9.05

Conclusion

In conclusion, the validated analytical method for CNZ and DMH exhibited excellent performance across all validation parameters, including linearity, precision, accuracy, sensitivity, and robustness. The system suitability results confirm good chromatographic separation, while recovery and repeatability studies show the method's accuracy and precision. Additionally, the method demonstrated sufficient sensitivity for trace analysis, as indicated by the low LOD and LOQ values. Forced degradation studies highlighted the method's capability to handle degraded samples, making it suitable for stability testing. Overall, this method is reliable for the routine quality control of CNZ and DMH in tablet formulations and can be employed for various analytical purposes, including stability studies and quantification of drug content.

References

1. Gupta, V., & Agarwal, S. (2011). Development and validation of a stability-indicating HPLC method for estimation of cinnarizine and dimenhydrinate in tablet dosage forms. Pharmaceutical Analysis, 2(4), 183-189.
2. Shetty, S. K., & Rao, R. S. (2014). Development and validation of a stability-indicating method for the simultaneous estimation of cinnarizine and dimenhydrinate in bulk and pharmaceutical formulations. International Journal of Pharmaceutical Sciences and Research, 5(7), 2789-2794.
3. Rajendran, S., & Thangavel, S. (2017). A stability-indicating HPLC method for determination of cinnarizine and dimenhydrinate in the presence of their degradation products. Journal of Chromatographic Science, 55(9), 923-930.
4. Saurabh, S., & Jain, A. (2020). Development of an HPLC method for the simultaneous determination of cinnarizine and dimenhydrinate in pharmaceutical formulations. International Journal of Drug Development and Research, 12(2), 21-28.
5. ICH (International Conference on Harmonisation). (2003). Stability testing of new drug substances and products. ICH Q1A(R2) Guidelines.
6. Jain, V., Shah, V.K. & Jain, P.K. (2019) HPLC method development and validation for the estimation of esomeprazole in bulk and pharmaceutical dosage form. Journal of Drug Delivery and Therapeutics, 9, 292–295.

7. Jain, P.K., Tyagi, C.K. & Dayaramani, R. (2021) Bioanalytical method development and validation for the estimation of saxagliptin in marketed tablet formulation. Journal of Pharmaceutical Research International, 33, 559–565.
8. Jain, P., Jain, A., Maliwal, D. & Jain, V. (2010) Development and validation of spectrophotometric and RP-HPLC method for estimation of olmesartan medoxomil in tablet dosage form. International Journal of Pharma and Bio Sciences, 1, 1–7
9. .Jain, P., Jain, A., Maliwal, D. & Jain, V. (2010) Development and validation of spectrophotometric and RP-HPLC method for estimation of olmesartan medoxomil in tablet dosage form. International Journal of Pharma and Bio Sciences, 1, 1–7.
10. Jain, P.K., Tyagi, C.K. & Dayaramani, R. (2021) Bioanalytical method development and validation for the estimation of saxagliptin in marketed tablet formulation. Journal of Pharmaceutical Research International, 33, 559–565
11. Jain, V., Shah, V.K. & Jain, P.K. (2019) HPLC method development and validation for the estimation of esomeprazole in bulk and pharmaceutical dosage form. Journal of Drug Delivery and Therapeutics, 9, 292–295.