An Innovative Method For Estimation Of Metformin HCl and Acarbose in Pharmaceutical Products and Seperation of Metformin Impurities By RP-HPLC

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Abstract : A Simple, rapid, cost effective, stability indicating RP-HPLC method has been developed for separation of Metformin HCl, its related impurities and Acarbose. Validated the method for simultaneous estimation of Metformin (MF) and Acarbose (ACB) in its novel combination of tablet formulation with Metformin 500 mg and Acarbose 50 mg. Metformin HCl is an orally-administered biguanide, anti-hyperglycemic agent, used in the management of non-insulin dependent diabetes mellitus. Acarbose is an oligosaccharide, used orally for the treatment of type 2 diabetes mellitus. The separation was achieved by using isocratic mobile phase consisting of mixture of phosphate buffer : acetonitrile (27:73 v/v), using Hypersil APS-2 column, (250 x 4.6 mm x 5µm) column at flow rate 2.0 mL/min. The detection was carried out at 210 nm with 20 µl of injection volume. The column temperature was maintained at 35 °C. The retention time (RT) of MF, its related impurities and ACB were found to be RT 2.6 min for 1-Cynogaunidine (RC A), 6.0 for Metformin (MF), 8.5 for 1-Methylbigaunidine (RC B), 10.4 for N,N-Dimethyl-1,3,5-triazine-2,4,6-triamine(RC C), and 12.2 min for Acarbose (ACB). The approach was found to be linear with the concentration of 5-25 µg/ml and 2.5-15µg/ml and correlation coefficient was 0.999 for MF and ACB respectively. The assay of estimated compounds was found to be 99.19% and 99.08% w/v and mean accuracy 100.66%, 101.59% for MF and ACB respectively. The developed method was validated as per ICH guidelines. The degradation products were well resolved from main peak. The validation was performed for various parameters like specificity, linearity, precision, accuracy and robustness studies. The method was found to be capable for simultaneous quantification of Metformin and Acarbose in its combination drug.
Key words : Metformin HCl, 1-Cynogaunidine, 1-Methylbigaunidine, N,N-Dimethyl-1,3,5-triazine-2,4,6-triamine, Acarbose, development, Degradation, Validation, USP, ICH guidelines.

Introduction

Metformin HCl (MF), molecular formula$C_4H_{11}N_5 \cdot$ HCl(Imidodicarbonimidic diamide, *N,N*-dimethyl-, monohydrochloride (Figure 1); also known as 1,1-Dimethylbiguanidemonohydrochloride) is an orally administered biguanide, antihyperglycemic agent, used in the management of noninsulin dependent diabetes mellitus[1-2].
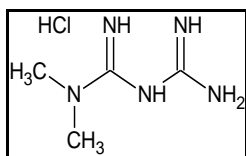

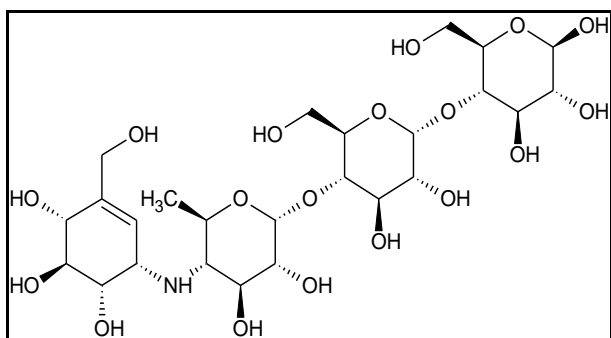**Figure 1: Structure of Metformin HCl**

Figure 2: Structure of Acarbose

It is very soluble in water, soluble in methanol and practically insoluble in methylene chloride. It has pKa of 5.1. ACB tablets are available in dose strength of 25 mg, 50 mg and 100 mg tablets (Glucobay®, Precose®, Manufactured by Bayer Healthcare)[6].

This multiple drug therapy has better healing properties along with improved patient acceptability[7-9].Oral hypoglycemic combination therapy is a better approach for management of glycaemia in the diabetic patients, hence we were decided to determine MF and ACB in single formulation. Combined therapy of metformin and Acarbose appears to be more efficacious than metformin or acarbose monotherapy Acarbose[10-12]. Literature search reveals that Acarbose has potential clinical utility as add-on therapy in the treatment of overweight patients with type 2 diabetes inadequately controlled with MF[13]. This combination formulation is available in Indian market with name of Glucobay M 50 (Metformin HCl 500 mg Acarbose 50 mg) tablets manufactured by Bayer Healthcare Limited, India.

Simultaneous determination of MF and ACB by HPLC method in this combination formulation was difficult due to fact that ACB being a sugar moiety has very less activity in terms of absorption at UV spectrum as compared to MF, which is a biguanide compound, and MF related substances were challenging to separate from ACB. Conjugation effect can be responsible for high intensity of absorbance in the MF and the same effect is absent in Acarbose. In addition to this ACB is in lower dose strength (50 mg) as compared to MF (500 mg) in the formulation[14].Literature survey was revealed many methods for determination of MF[15] and ACB[16] in their respective formulations. Few methods have been reported yet for the specified combination formulation[17]but has and not shown the separation of MF and related substance RC A, RC B and RC C separation from ACB. Objective of the proposed work was to develop, performed degradation and validate HPLC method for determination of MF and ACB in its combination formulation. An innovative method for separation of Metformin (MF), its related impurities and Acarbose (ACB) and simultaneous estimation of MF and ACB have been developed by RP-HPLC and validated according to ICH guidelines.

Materials and Methods

Materials and Reagents

Working standard of ACB, formulation excipients and test samples (Glucobay M 50 tablets) obtained from Bayer Healthcare Limited India. MF and impurities obtained from Auro Laboratories Limited India. HPLC grade Acetonitrile, AR grade Potassium dihydrogen orthophosphate and disodium hydrogen phosphate

dihydrate purchased from Merck India Limited. High purity HPLC water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system.

Instrumentation and Chromatographic Conditions

HPLC system (Waters 717 plus autosampler HPLC system USA) consisting of pump 515, Injector and 2487 dual wavelength absorbance UV detector were used for analysis. Chromatographic data was acquired using Empower software. Hypersil APS-2 (250 x 4.0 x 5 µm) (Thermoelectron, USA) column was used as a stationary phase. The isocratic mobile phase consisting of mixture of buffer (4.4 mM potassium dihydrogen phosphate and 1.96 mM of disodium hydrogen phosphate): Acetonitrile (27:73 v/v) was used throughout the analysis. The flow rate of the mobile phase was 2.0 ml/min. The detection was carried out at wavelength of 210 nm. The column temperature was kept at 35°C and injection volume at20 µl.

Sample solution preparation for Assay

Twenty tablets were weighed accurately and crushed to powder with aid of mortar. Powdered tablets weighed and transferred to volumetric flask, water was added, and flask was sonicated for 1 hour. The solution was allowed to stand for 10min and diluted to get the concentration 1.0 mg/ml and 0.10 mg/ml for MF and ACB respectively. Centrifuged the solution at 5000 rpm for 5 minutes and supernatant was filtered with 0.45µm membrane filter and injected.

Organic impurities and standard solution preparation

Standard solution for MF and ACB was prepared and diluted to get the concentration 1.0 mg/ml and 0.10 mg/ml respectively. Spiked standard solution prepared with spiking of MF related substance RC A, RC B, RC C to ensure the separation of impurity from MF and ACB.

Analytical Method Validation

This method was validated as per ICH Q2 (R1); 2005 guideline [18]for linearity, precision, intermediate precision, accuracy and robustness. The specificity of the method was determined by injecting the sample solution, impurity mixture with standard solution, drug product and degradation solution. This ensure that method is capable of separating impurity and active pharmaceutical ingredients without any interference.

System Suitability

The solution containing mixture of MF (1000 µg mL$^{-L)}$) and ACB(100µg mL^{-L}) was injected in replicates to check the system suitability criteria. The criterion includes the following parameters like percentage RSD, resolution, tailing factor and theoretical plates were checked and confirmed through this study. The criterion is as follows: resolution should be greater than 1.5, RSD value should not exceed 2.0%, the tailing factor is in between 0.7–1.5.

Linearity

Linearity is used to perform to ensure the method capability for detection with lower to higher range of concentration with linear response. Five standard solutions were prepared for the linearity test in the range of 0.25-2.0 mg/ml of MF and 0.025-0.20 mg/ml of ACB. Each solution was injected in three replicates and linear was calculated from calibration curve. The limit of detection (LOD) of MF and ACB was determined from the regression data of calibration curve by using formula as LOD= 3.3(SD)/S, whereSD is the average residual standard deviation and S is slope of the calibration curve. Limit of quantitation(LOQ) was calculated using the formula LOQ= 10(SD)/S.

Accuracy

The accuracy of the method is performed to check the capability of method for giving the accurate results with variation of sample concentration or by addition of known amount of standard solution to the sample, at three different level and calculated against standard solution. In this study, accurately weighed fined tablet powder equivalent to three concentration levels 80%, 100% and 120% of the label claim. The result was calculated with true value obtained from tablet solutions. The accuracy results were reported as percentage recovery with difference in results from actual concentration and recovered concentration. Acceptance criteria of the result was kept as mean recovery should be in the range of 98% to 102%, and %RSD should be not more than 2.

Precision

Precision study is performed to measure the degree of repetition and reproducibility under given test condition. Precision of the method was checked by carrying out six independent assays of test samples against standard. %RSD criteria is used to evaluate the method. In this study, average weight of 20 tablets were taken, grind finely, accurately weighed the tablet powder equivalent to the standard concentration. six preparation of MF and ACB combination tablet was performed. Intermediate precision was performed by analyzing the samples by different analyst on different day with different instrument.

Robustness

Robustness is susceptibility of analytical method towards deliberate changes in the chromatographic conditions. The flow rate of the mobile phase was changed ($\pm$0.2 ml/min) from 2.0 ml/min to 1.8 ml/min and 2.2 ml/min. The organic strength was varied by $\pm$2% units of minor components. Standard solution was injected in six replicates for each change. Respective peak areas, dilution factors, sample and standard weights were considered to quantitate the amount of MF and ACB in mg per tablet.

Specificity

Stress studies were carried out for drug product to identify the possible degradation products. This study helps to establish the degradation pathways and the inherent stability of the molecule.

As per ICH guidelines[19], degradation studies were performed under various stressed conditions to make available of the specificity and stability indicating properties of method. The forced degradation study of the method was carried out for aqueous hydrolysis, acid hydrolysis with 0.50N HCl, oxidative degradation with 3% peroxide, base hydrolysis with 0.50N sodium Hydroxide, thermal degradation at 105°c for 1 day. Photo stability was conducted with the sample exposed to UV and Sun light. Sample was kept with covered and uncovered with aluminum foil. In aqueous degradation, 100.0 mg of sample was diluted to 10 ml and refluxed at 60°c for 7 h. In acid hydrolysis, 100.0 mg of sample was dissolved in 10 ml 0.5 N HCl, and sample was refluxed at 60°c temperature for 1 day. Before the analysis, sample solution was neutralized with 1.0 N of NaOH solution and appropriate dilution was given to achieve the test concentration. Oxidative degradation was carried out by using 3.0% of Hydrogen Peroxide, about 100.0 mg of sample was dissolved in 10 ml of 3.0% of Hydrogen Peroxide and made up to the mark with diluent. The solution was analyzed after appropriate dilution with mobile phase. For base hydrolysis, 100.0 mg of sample was dissolved in 10 ml of 0.50 N sodium Hydroxide and sample was the refluxed at 60°c temperature for 1 day. Before analysis, sample solution was neutralized with 1.0N Acetic acid, then it was diluted to get the test concentration. In thermal degradation study, 1.0 gm of the sample was spread as a uniform thin layer in Petri dish and then kept in the oven at 105°c for 1 day. Finally, sample was diluted to get final test concentration. In photo stability study, 1.0 gm of the sample was spread as a uniform thin layer in Petri dish and kept under UV and Sun light for 7 h. Finally, sample was diluted to get final test concentration. In Humidity, 1 gm of sample was kept under 85°/85% RH in humidity chamber for 1 day, then sample solution was prepared and diluted to get final test concentration.

Assay of formulation sample

Applicability of developed procedure was evaluated by analyzing three commercial lots of the Glucobay M 50 tablets for content of MF and ACB. Standard solution was prepared as reference and assay of sample solutions were calculated against standard solution.

Results and Discussion

Method Development and Optimization

Separation of MF impurity from MF and ACB was difficult task. Different HPLC method described in literature were tried, but unable to get desired results. There were lot of difference in concentration of MF and ACB. To overcome this problem, series of sample solution were prepared and tested. Standard and test solution concentration having concentration of MF1.0 mg/ml and ACB 0.1 mg/ml was found to be optimum in terms of extraction during the sample preparation and peak shape. Sonication time was optimized with respect of

complete extraction of ACB, MF and excipients. Sonication time of 60 min with intermittent shaking was found to be appropriate in terms of complete extraction. To balance the dilution factor, the injection volume 10 µl was change to 20 µl. Effect of the composition of the mobile phase on the retention time of MF and ACB was thoroughly investigated. Mobile phase composition of Phosphate buffer: Acetonitrile (27:73 v/v) was found to be optimum to separate the MF and its related compound peaks from ACB peak. Column temperature had effect on the ACB peak symmetry. Temperature below 30°C results peak symmetry factor more than 2, hence column temperature of 35°C was found to be optimum to get the peak symmetry factor less than 1.5. Mobile phase flow rate of 2 ml/min was found to be optimum for separation of MF, RC A, RC B, RC E and ACB with satisfactory resolution. The chromatogram was recorded at 210 nm. The elution order was found to be 2.6 min of RC A, 6.0 min of Metformin (MF), 8.5 min of RC B, 10.4 min of RC C, and 12.2 min of Acarbose (ACB) at optimized chromatographic condition (Figure3). Developed procedure found to be specific for MF and ACB for assay determination from any interference.
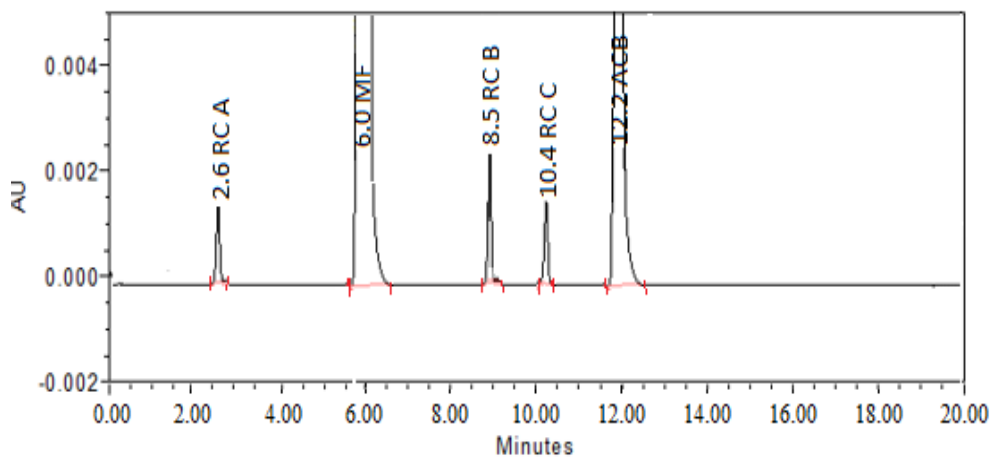

Figure 3: Chromatogram for Standards of MF, ACB and MF related substance

Method Validation

Method validation was performed as per ICH and USP General Chapter <1225>, and specificity, Linearity, Accuracy, Precision, and Robustness studies were performed. The HPLC chromatogram recorded for the mixture of the drug excipients revealed no peak. The chromatogram recorded for mixture of MF, its related impurities, and ACB, it shows distinguishing peaks for the two actives along with the peak of MF related substance as shown above in (Figure3). All the degradation solution shows that there was no interference with MF and ACB (Figure4). Hence the method was found to be specific.
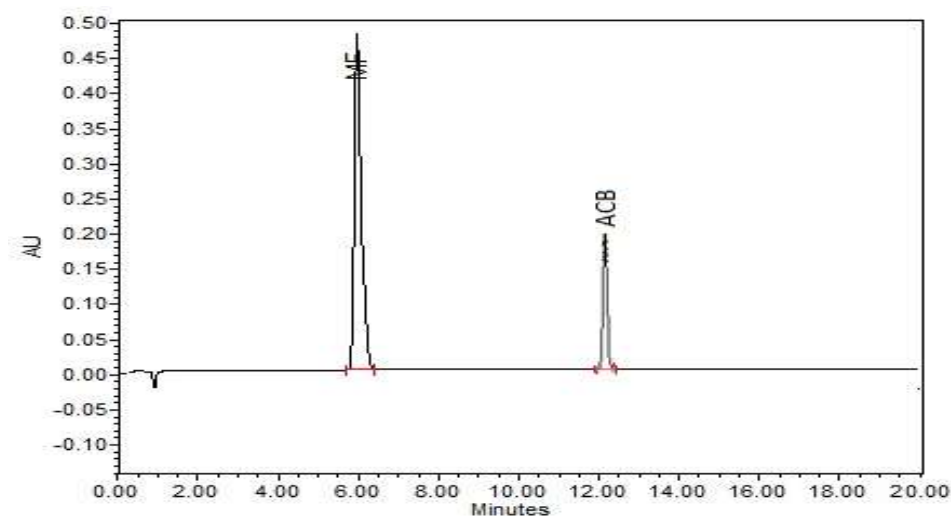

Figure4: Standard Chromatogram of Metformin and Acarbose

Linearity

Linearity study was performed and curve with five points were constructed covering a concentration range 0.25–2.0mg/ml of MF and 0.025-0.20mg/ml of ACB. Linearity regression curves were plotted by area versus concentration and data was given in Table-1.

Table 1: Linearity Regression Data for Calibration Curves

Parameter (units)	MF	ACB
Linearity range (mg/ml)	0.25-2	0.025-0.2
Slope	13217.4	7253.5
Intercept	3242.6	474.3
Correlation Coefficient (r^2)	0.9997	0.9999

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of MF and ACB in the tablets from the sample solution. Sample solution were prepared for the concentration of 80%, 100% and 120% of actual concentration by weight variation method. The mean recovery data of MF and ACB in real sample were within the range.

Precision

The precision study and intermediate precision was performed for tablet sample. Six preparation was performed at each level at 100% level and %RSD was calculated for six determination. The results were well within the acceptance limit RSD<2% indicating a good system and method precision.

Robustness

In robustness study, all deliberately varied conditions, the RSD of peak areas of MF and ACB found to be well within the acceptable limit of 2% and the symmetry factor was found to be <1.5. The summery of validation results are given in Table 2.

Table 2: Summery of Validation Parameter

Parameter (Units)	MF	ACB
Linearity Range (mg/ml)	0.25-2	0.025-0.2
Correlation coefficient	0.9997	0.9999
Recovery (%)	100.66	101.59
Precision (%RSD)		
Interday (n=6)	0.50	0.79
Intraday (n=6)	0.49	0.81
Robustness	Robust	Robust
Retention time ± Allowable time (min.)	6.02±0.2	12.2±0.2
Resolution	NA	5.54
Theoretical plates	6021	3200
Tailing factor (symmetry factor)	1.15	1.21

Degradation behavior of the drug product

During all above degradation studies, it was observed that no major unknown degradation was found, and in all above degradation condition, degradation was 1% to 7% for MF, and 0.4% to 5% in ACB. Only know impurities were found to be increased and degradation was calculated based on assay obtained after final analysis. The data obtained from forced degradation studies were proved that method was specific for determination of MF and ACB in combination tablet. Degradation chromatograms were given in (Figure4) and results were given in Table 3.
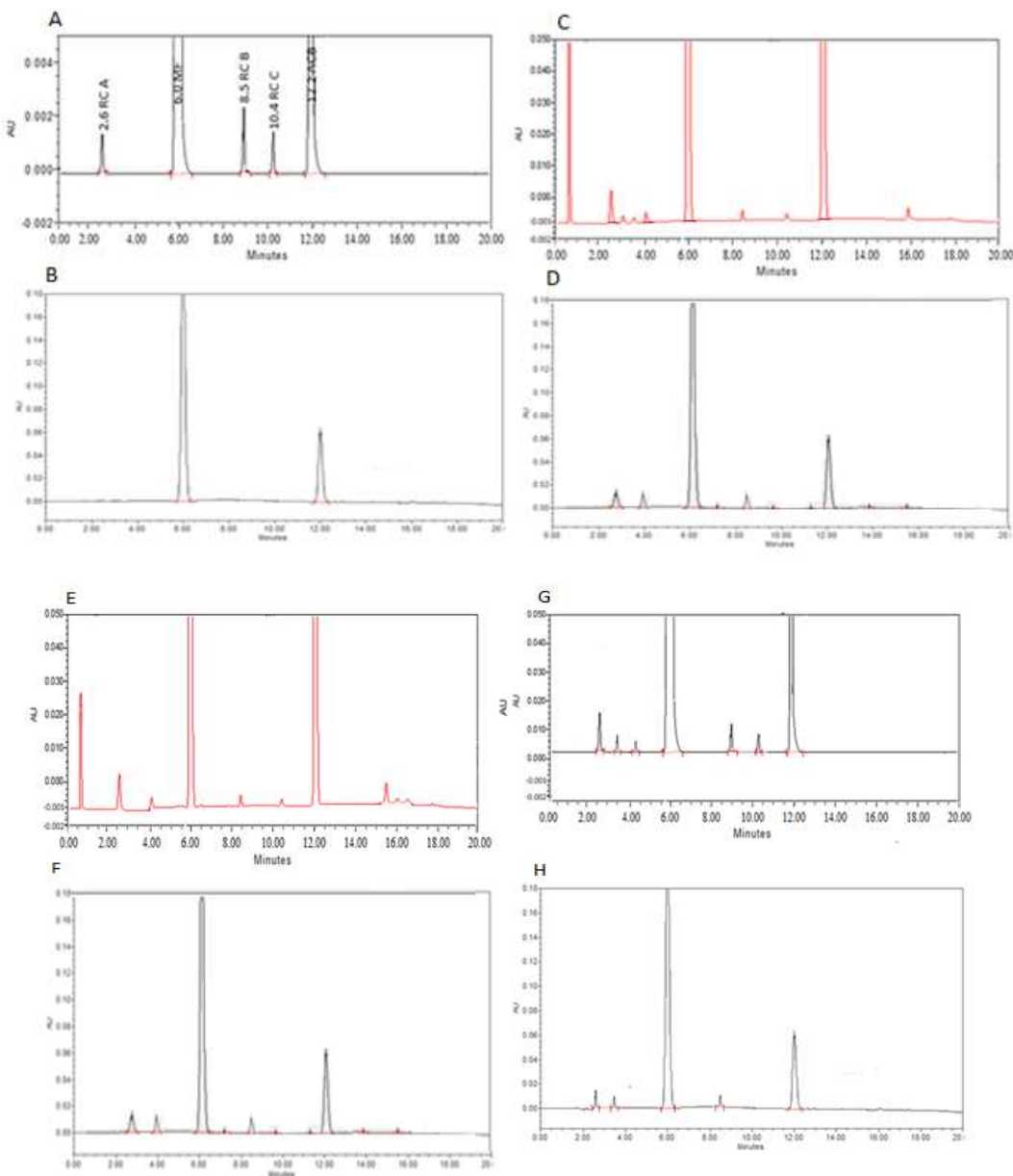
Table 3: Summery Of Degradation Studies For Mf And Acb

Degradation Condition	Time (h/day)	%Degradation	
		MF	ACB
Neutral Hydrolysis	6 h	**	**
Acid, 0.5 N HCl (heated at 60°)	1 day	5.84	4.94
Base, 0.5 N NaOH (heated at 60°)	1 day	6.23	4.97
Oxidative, 3% W/V H_2O_2(heated at 60°)	1 day	6.84	4.29
Thermal, (heated at 105°)	1 day	4.59	4.72
Humidity, 85°/85% RH	1 day	5.52	5.12
Direct sunlight (photolysis)	7 h	**	**
UV degradation at 256 nm	7 h	**	**

**No degradation observed

Figure 4: Degradation Chromatograms of Metformin Acarbose Tablet

A. System suitability of MF, its impurities and ACB, B. Aqueous sample, C. Peroxide degradation, D. Thermal degradation, E. Acid degradation, F. Base degradation, G. Humidity degradation, H. UV degradation

Market sample analysis

Validated method was applied for determination of Acarbose in the three commercial lots of the combination formulation. The results were presented in Table 4. The results were complied the label claim very well, reflecting the reproducibility of the proposed method.

Table 4: Assay of MF and ACB In Glucobay M-50 Tablets

MF			ACB		
Batch	Label claim in mg	% Assay	Label claim in mg	% Assay	
1	500	99.82	50	99.80	
2	500	99.16	50	99.86	
3	500	98.58	50	97.58	

Conclusion

The developed method was found to be simple, specific, accurate, precise and robust. The approach was easy and economical, that could be efficiently applied for simultaneous estimation of both MF and ACB in combination tablet. Also, it provides information about impurities available due to MF simultaneously. The proposed method can be utilized for routine analysis and in quality control laboratory for determination of MF and ACB in its novel combination formulation available commercially.

Acknowledgement

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