Journal of Pharmaceutical Research Vol. 13, No. 3, July - September 2014 : 80-84

A VALIDATED STABILITY INDICATING METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCl AND ACARBOSE IN BULK AND ITS COMBINED TABLET DOSAGE FORM BY RP-HPLC

Nagaraju P* and Ramanakanth M V L
Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur - 522 002, Andhra Pradesh, India.

Received on: 03.09.2014 Revised: 27.09.2014 Accepted: 30.09.2014

ABSTRACT
The current work explains simple, accurate, rapid and precise isocratic reverse-phase high-performance liquid chromatographicstability indicating method for simultaneous determination of metformin HCl and acarbose in bulk and combined dosage form. The chromatographic separation was carried out on Zorbax C-18 analyticalcolumn (4.6×250 mm; 5 µm) with a mixture of methanol: 0.1% O- phosphoric acid 60:40(v/v) as mobile phase; at a flow rate of 1.2 ml/min. UV detection was performed at 239 nm. The retention times were 2.218 min and 2.972 min for metformin HCl and acarbose respectively. Calibration plots were linear over the concentration range of 400-1200 µg/ml for metformin HCl and 40-120 µg/ml for acarbose. The method was validated for accuracy, precision, linearity and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Degradation studies were performed by subjecting the sample to various stressed conditions of acid, alkali, peroxide, thermal and photolytic studies.

Keywords: *Metformin HCl; Acarbose; RP-HPLC; Stability indicating method.*

INTRODUCTION
Metformin hydrochloride (MET) (Fig.1) is chemically 1,1-Dimethylbiguanide hydrochloride[1] and a well known oral anti-diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes. Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis). Acarbose (ACR) (Fig.2) is chemically O-4,6-dideoxy-4-[[(1S,4R,5R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose[2] and an anti-diabetic drug used to treat type 2 diabetes mellitus. It is a starch blocker, and inhibits alpha glucosidase, an intestinal enzyme that releases glucose from larger carbohydrates. It is composed of an acarviosin moiety with a maltose at the reducing terminus.

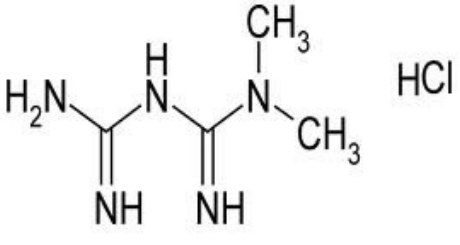

Fig 1: *Metformin HCl*

Literature survey reveals that few spectroscopic[3-6] and chromatographic[7-14] methods were reported for determination of MET and ACR in single and combination with other drugs. To the best of our knowledge hitherto there is no stability indicating RP-HPLC method for simultaneous determination of binary mixture containing MET and ACR. Therefore, an attempt has been made to develop a simple, accurate, rapid and reproducible stability indicating RP-HPLC method for simultaneous determination of MET and ACR in combined tablet dosage form and validated in accordance with ICHguidelines[15].

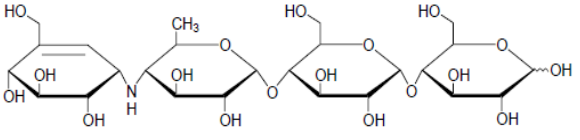

Fig 2 : *Acarbose*

MATERIALS AND REAGENTS
Pharmaceutical grade of MET and ACR working standards were obtained from Cipla Pvt Ltd. Mumbai. Commercially availableGlucobay-M (Bayer Pharmaceutical Pvt Ltd, Solan, Himachal Pradesh, India) tablets claimed to contain 500mg of MET: 50mg of ACR have been utilized in the present work. HPLC grade water from Rankem, HPLC grade methanol, hydrogen peroxide, O-phosphoric acid (OPA), 0.45 µm PVDF filters were acquired from Merck Ltd, Mumbai, India.

Chromatographic system and conditions
Separation was performed with Waters HPLC equipped with a pump-2695, auto sampler- 2707 and PDA detector-2998. Empower 2 software was applied for

***Correspondence :** email: pappulanagaraju@gmail.com Mob : 9985304304

data collection and processing. The separation was achieved on a Zorbax C-18 (4.6×250 mm, 5 µm) analytical column. The mobile phase consisted of methanol : 0.1% O-phosphoric acid 60:40 (v/v). The flow rate was 1.2 ml/min and UV detection was performed at 239 nm. The mobile phase was sonicated on ultrasonic bath for 15 min and was filtered through a 0.45 µ membrane filter. The injection volume was 10 µl and all the experiments were performed at ambient temperature.

Standard stock solution

Accurately weighed and transferred 100mg of MET and 10mg of ACR working standard into 50ml volumetric flask, added about 35ml of water and sonicated to dissolve it completely and made volume upto the mark with same solvent (stock solution). From this stock solution 4ml was transferred to 10 ml volumetric flask and made the volume to mark with water to obtain the final concentrations of 800 µg/ml of MET and 80µg/ml of ACR. Mixed well and filtered through 0.45 µ filter.

RESULTS AND DISCUSSION

Optimization of Chromatographic conditions

To develop a stability indicating method, different stationary phases like C_{18}(150mm, 250mm) column, cyano column, different mobile phases containing buffers like phosphate, ammonium acetate with different pH (3-7) were used. Our objective of chromatographic method development was to achieve peak tailing factor < 2, retention time between 2 to 5min along with resolution between MET and ACR > 2. The chromatographic separation was achieved using Zorbax C18 (250x4.6mm, 5µm) column, changing the composition of mobile phase and optimized the chromatographic method. To develop a stability indicating method assessing the effect of change of proportion, MET and ACR were well resolved from the degradation products using mobile phase composition of methanol:0.1% O-Phosphoric acid (60:40 v/v) at a flow rate of 1.2ml/min with detection at 239nm wavelength and injection volume 10µl. It was found that the peaks of MET (Rt 2.218min) and ACR (Rt 2.972min) were ideally resolved (fig 3). Resolution (Rs) between MET and ACR was found to be 6.68.

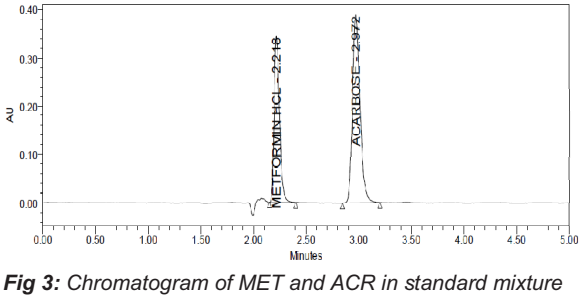

Fig 3: *Chromatogram of MET and ACR in standard mixture*

Analysis of tablet formulation

Twenty tablets of Glucobay-M (Bayer Pharma) each containing 500 mg of metformin HCl and 50mg of acarbose were weighed and crushed in glass mortar to obtain fine powder. The powder sample equivalent to 1000 mg of MET and 100 mg of ACR was transferred into 100ml volumetric flask and dissolved in 75ml of water HPLC grade. The flask was kept in ultrasonic bath for 15 min. The volume was adjusted to 100ml with water HPLC grade. The solution was filtered through 0.45µ filter. From the stock solution 2ml solution was pipetted out and transferred to 25ml volumetric flask and volume made up to the mark with water HPLC grade to get the concentration 800µg/ml of MET and 80µg/ml of ACR. The solution was injected into HPLC system (fig 4). The results of the assay of tablet formulation and its statistical validation data is given in Table 1.

Table 1: *Assay results of MET and ACR*

Drugs in Formulation	Label Claim	Amount found*	Assay %*
Metformin HCl	500 mg	495.05 mg	99.01
Acarbose	50 mg	49.69 mg	99.38

**Average of six determinations*

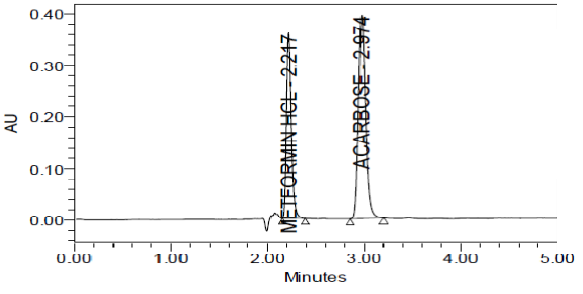

Fig 4: *Chromatogram of MET and ACR in sample mixture*

METHOD VALIDATION

The developed method was validated according to ICH guidelines. Method validation was carried out by system suitability, linearity, accuracy, precision, robustness and forced degradation studies.

System suitability

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the standard mixture solution at a concentration of 800µg/ml and 80µg/ml for MET and ACR respectively.

Linearity

Standard linearity curves were plotted against the concentration ranging from 400-1200µg/ml for MET and 40-120 µg/ml for ACR. Each concentration was injected six times into the HPLC system keeping the injection volume (10µl) constant. Linearity curves were plotted and the correlation coefficients were found to be 0.9998 for MET and 0.9997for ACR. The plots are shown in Fig 5 and Fig 6.

Accuracy

To study the reliability and suitability of developed method, accuracy was performed by recovery experiments, which were carried by standard addition method. A known amount of standard drug was added to

the pre-analyzed sample solution at 3 concentration levels i.e 80%, 100% and 120%. The resulting solutions were analyzed in triplicate at each level. The results are given in Table 2.

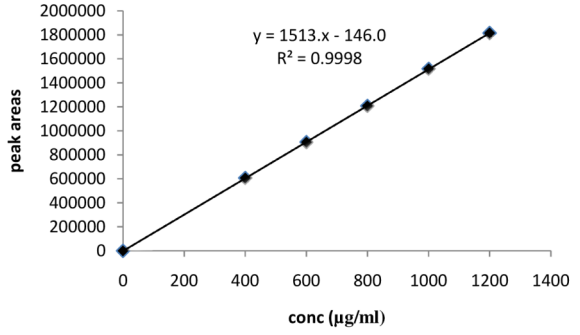

Fig. 5 : *Linearity plot of metformin HCl*

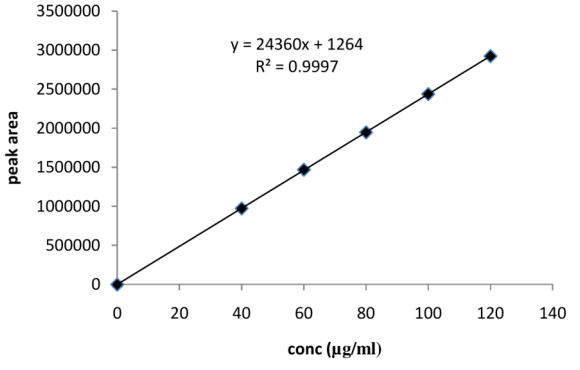

Fig. 6 : *Linearity plot of acarbose*

Table 2: *Accuracy results of MET and ACR*

Drug	Spiked level	Spiked concentration µg/ml	Obtained concentration* µg/ml	% Mean recovery
MET	80%	400+320	719.62	99.88
ACR	80%	40+32	71.86	99.56
MET	100%	400+400	799.61	99.90
ACR	100%	40+40	79.88	99.70
MET	120%	400+480	880.13	100.02
ACR	120%	40+48	88.05	100.08

**Average of three determinations*

Precision

Precision of assay was determined by system precisionand method precision. Every sample was injected six times. The repeatability of sample application and measurements for peak area were expressed in terms of %RSD and it was found to be less than 2, which indicates that the method is more precise.

Limit of Detection and Limit of Quantification

Limit of Detection (LOD) and Limit of Quantification (LOQ) were estimated from signal-to-noise ratio of 3:1 and 10:1 respectively by injecting a series of dilute solutions of known concentrations. The results are given in Table 3.

Table 3: *Validation parameters of MET and ACR*

Parameter	Metformin HCl	Acarbose
Retention time (min)	2.218	2.972
Tailing factor	1.32	1.23
USP Resolution	-	6.68
Plate count	9518	8377
Linearity range (µg/ml)	400-1200	40-120
Correlation coefficient	0.9999	0.9998
LOD(µg/ml)	4.22	0.42
LOQ(µg/ml)	15.88	1.44
System precision (%RSD)	0.28	0.14
Method precision (%RSD)	0.28	0.15

Robustness

To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included variation of flow rate, mobile phase composition. The result showed minute variations and the method was proved to be robust. The results are shown in Table 4.

Table 4: *Robustness results for MET and ACR*

Condition%	Assay	
	Metformin HCl	Acarbose
Change in flow rate:		
• Flow rate at 1.0ml/min	100.31	99.53
• Flow rate at 1.1ml/min	100.23	99.84
Change in mobile phase composition:		
• Methanol : Buffer (58:42)	99.81	100.18
• Methanol : Buffer (62:38)	100.11	99.12

Forced degradation studies

Forced degradation studies which include acidic, alkaline, peroxide, thermal, and photolytic conditions were carried out by heating and refluxing using a heating mantle with temperature control, which was done as per ICH guidelines Q1B[16]. Sample solutions were prepared to attain concentration of 800µg/ml for MET and 80µg/ml for ACR in 0.1N HCl, 0.1N NaOH and 30% H_2O_2 separately, then heated for 2hour at 60°C and sonicated for 30 min. Thermal degradation was performed by placing the sample in petri plate and kept in hot air oven for 6hour at 100°C and photolytic degradation was studied by subjecting the powdered sample to sunlight for 48hour, then both the samples were diluted to get the desired concentrations of the drugs. Different aliquots of solutions were injected and chromatograms (Fig 7- Fig 11) were obtained. The % degradation of both the drugs were calculated and reported in Table 5.

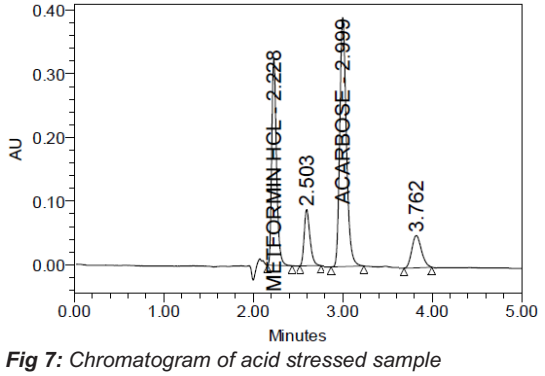

Fig 7: *Chromatogram of acid stressed sample*

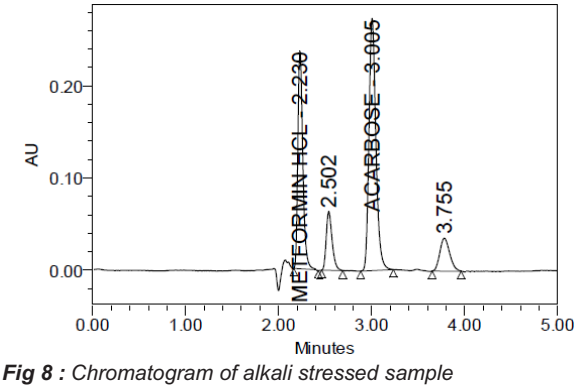

Fig 8 : *Chromatogram of alkali stressed sample*

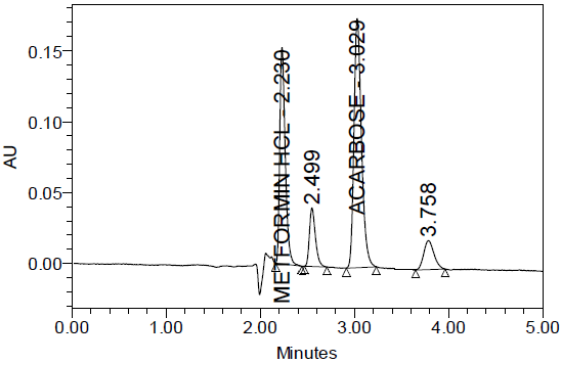

Fig 9 : *Chromatogram of peroxide stressed sample*

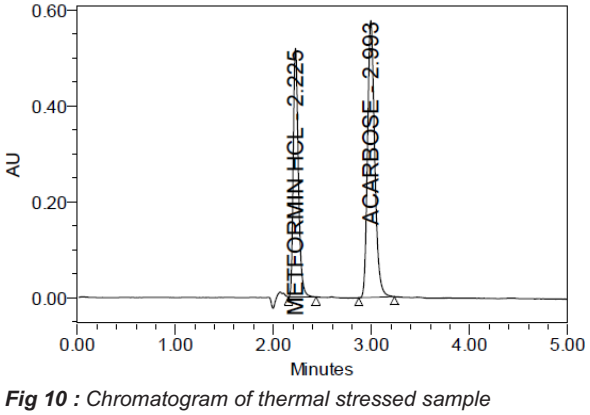

Fig 10 : *Chromatogram of thermal stressed sample*

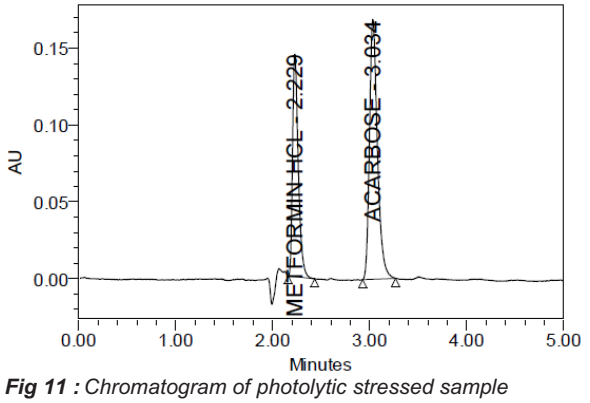

Fig 11 : *Chromatogram of photolytic stressed sample*

Table 5: *Forced degradation results for MET and ACR*

Stress condition	Metformin HCl		Acarbose	
	% Assay	% Degradation	% Assay	% Degradation
Acid	93.24	5.86	93.41	6.29
Alkali	92.68	6.42	93.81	5.89
Peroxide	92.59	6.51	93.68	6.02
Thermal	94.28	4.82	95.57	4.13
Photolytic	95.25	3.85	96.57	3.14

CONCLUSION

The developed HPLC method is simple, specific, accurate and precise for the simultaneous determination of MET and ACR in combined tablet dosage form. The developed method provides good resolution between MET and ACR. It was successfully validated in terms of system suitability, linearity, precision, accuracy, LOD, LOQ and robustness in accordance with ICH guidelines. The stability tests showed that these two drugs were significantly unstable and the degraded products were clearly separated from main peaks. Thus the described method is suitable for routine quality control analysis of these drugs in API and pharmaceutical formulations.

ACKNOWLEDGEMENTS

The authors are thankful to Cipla Pvt Ltd, Mumbai, for providing metformin HCl and acarbose as gift samples for this work, and also thankful to Hindu College of Pharmacy, Guntur for providing required facilities for research work.

REFERENCES

1. The Indian Pharmacopoeia. Vol II, Ghaziabad: The Indian Pharmacopoeia Commission, 2010, p 740.
2. The Indian Pharmacopoeia. Vol II, Ghaziabad: The Indian Pharmacopoeia Commission, 2010, p 60.
3. Mubeen G and Khalikha Noor. Spectrophotometric Method for Analysis of Metformin Hydrochloride. Indian J Pharm Sci. 2009; 71(1): 100–102.
4. Arayne MS, Najma Sultana, Zuberi MH, and Siddiqui FA. Spectrophotometric Quantitation of Metformin in Bulk Drug and Pharmaceutical Formulations using Multivariate Technique. Indian J Pharm Sci. 2009; 71(3): 331–335.
5. PatilSudarshan S, Bonde, CG. Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV - visible spectroscopy. International Journal of ChemTech Research. 2009; 1(4): 905-909.
6. Kumar G, Juyal V, Badoni PP, Kumar S, Rawat MSM. Spectrophotometric Method Development of AcarboseFrom Bulk And In Its Tablet Dosage Form. Journal of Pharmacy Research. 2009; 2(10): 1595
7. Saeedarayne M, Najma Sultana and Hashim-Zuberi M. Development and validation of RP-HPLC method for the analysis of Metformin. Pak. J. Pharm. Sci. 2006; 19(3): 231-235.

8. Bondea SL, Bhadanea RP, AvinashGaikwad, et al. A Simple And Sensitive Method For Determination Of Metformin And Sitagliptin In Human Plasma Using Liquid Chromatography And Tandem Mass Spectrometry. Int J Pharm Sci. 2013; 5(3): 463-470.
9. LakshmanaraoA, et al. Simultaneous estimation of Metformin and Vildagliptin in solid dosage form by stability indicating RP-HPLC method. IntRes JPharm. 2013; 4(1): 122-128.
10. Vijay Kumar M, Muley PR. Reverse phase HPLC determination of acarbose in solid dosage forms. The Indian pharmacist. 2005; 4(37): 63-66.
11. Dhaneshwar SR, Salunkhe JV, Bhusari VK. Validated HPTLC Method for Simultaneous Estimation of Metformin Hydrochloride, Atorvastatin and Glimepiride in Bulk Drug and Formulation. J Anal Bioanal Tech. 2010; 1: 109.
12. Shailaja B. Jadhav, Swati K. Kupkar, Deepali L, et al. Development and Validation of RP-HPLC and HPTLC Methods for Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Bulk and Dosage form. Ind J Pharm Edu Res, 2013; 47(1): 13-16.
13. Raut BB, KolteBL, DeoAA, BagoolMA and ShindeDB. Quantification of Acarbose in Human Plasma by Liquid Chromatography—Electrospray Tandem Mass Spectrometry. JLiqChr. 2004; 27(11): 1759-1768.
14. Raniah Q, Gabr, Raj S, Padwal and Dion R. Brocks. Determination Of Metformin In Human Plasma And Urine By High Performance Liquid Chromatography Using Small Sample Volume And Conventional OctadecylSilane Column. J Pharm Pharmaceut Sci. 2010; 13(4): 486-494.
15. ICH, Q2B- Validation of Analytical Procedures: Methodology, International Conference on Harmonization, 1996; 137.
16. ICH, Q1B- Stability Testing: Photostability Testing of New Drug Substances and Products, 1996.