AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

VALIDATED RP-HPLC FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN PHOSPHATE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM.

Shyamala.M*[1], Mohideen.S[1], Satyanarayana .T[1], Ch.NarasimhaRaju[1], Suresh Kumar.P[1], Swetha.K[1]

1. Browns college of Pharmacy, Khammam, Andhra Pradesh.

ABSTRACT

Rapid and accurate High performance liquid chromatography method is described for Simultaneous estimation of Metformin Hydrochloride and Sitagliptin Phosphate from the combination tablet dosage form. The separation of two drugs was achieved on HYPERSIL (250 x 4mm i.d) 5µ column. The mobile phase consists of Acetonitrile and phosphate buffer in the ratio of 45:55. The detection was carried out at a wavelength 260nm. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution. The linear ranges for Metformin Hydrochloride and Sitagliptin Phosphate were 20-120µg/mL, 2-12µg/mL respectively with good recoveries i.e. 99.16% to 99.89%.

Keywords: Metformin hydrochloride, Sitagliptin phosphate, High performance liquid chromatography.

*Corresponding Author Email: shyamala.mudavath@gmail.com

Received 12 August 2011, Accepted 23 August 2011

Please cite this article in press as: Shyamala M. *et al.*, Validated RP-HPLC For Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in tablet dosage form. American Journal of PharmTech Research 2011.

INTRODUCTION

Metformin hydrochloride and Sitagliptin phosphate are available in tablet dosage form in the Ratio of 1:10. Chemically Metformin is N, N – dimethyl imidodicarbonomidicdiamide has anti diabetic activity. Sitagliptin is 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate has hypoglycaemic agent. Metformin is official in IP[1] and USP[2], while Sitagliptin is not official in any pharmacopoeias. Literature survey reveals, UV[3], HPLC[4] methods for analysis of Metformin as single and combined dosage forms with other drugs and UV[5], HPLC[6] methods for analysis of Sitagliptin as single component systems. There are no reported methods for analysis of both drugs in combination. This paper presents simple, rapid, accurate and economical methods for simultaneous analysis of Metformin hydrochloride and Sitagliptin phosphate in tablet dosage form.

MATERIALS AND METHODS

Instrument

The HPLC system used was Shimadzu with model no SPD-20A equipped with UV detector source of deuterium lamp. The chromatogram was recorded at and peaks quantified by means of PC based Spinchrome software.

Solvents used

Acetonitrile and Water for buffer HPLC grade from as a solvent in the study and obtained from MERCK Company, Mumbai.

Prepation Standard Stock Solutions

Stock solution was prepared by transferring 50mg of Metformin Hcl and 0.5 mg of Sitagliptin in 50ml volumetric flask in 50ml volumetric flask.

Sample preparation

Powder equivalent to 50mg of Metformin Hcl was weighed and transfer into 50ml volumetric flask, mobile was added to it, sonicate and filterd through Whatmann filter paper, resulting solution was diluted to 80mcg/ml was injected into HPLC system.

Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reverse phase HYPERSIL (250 x 4mm id) 5µ column. Mobile phase was made up of acetonitrile : phosphate buffer(0.02M of potassium di hydrogen phosphate and 0.03M of dihydrogen potassium in 550ml) in a ratio of 45:55. The mobile phase was filtered, degassed before use. The flow rate

was adjusted to 1ml/min. the detector wavelength was set at 260nm. The injector volume of the standard and sample was 20µl.

Method validation

The method was validated as per International Conference on Harmonization (ICH) guidelines[7,8].

System Suitability Tests (SST)

Once a method or system has been validated the task becomes one of routinely checking the suitability of the system to perform within the validated limits. The simplest form of an HPLC system suitability test involves a comparison of the chromatogram trace with a standard trace. This allows a comparison of the peak shape, peak width, and baseline resolution. Alternatively these parameters can be calculated experimentally to provide a quantitative system suitability test report such as number of theoretical plates (efficiency), Capacity factor, Separation (relative retention), Resolution, Tailing factor. These are measured on a peak or peaks of known retention time and peak width.

Linearity

Linearity of the method was determined by mean of calibration graph using an increasing amount of each analyst. Linearity was evaluated by visual inspection of a calibration graph. At least three concentration levels were tested in agreement to ICH. The slope, intercept was reported as required by ICH. LOD and LOQ were estimated from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the intercepts of the regression lines done in the range of the detection limit.

Accuracy

The accuracy of the method was measured by recovery studies and ascertained by standard addition method. A known amount of pure drug at three different levels i.e. 80 %, 100 %, and 120 % was added to pre-analyzed sample solutions and total concentration was determined using the proposed method.

Precision

Precision was investigated at three levels, intra-day, inter-day, and reproducibility. The intra- and inter- day variability were assessed by using standard drug solution at three different concentration. Intra-day precision was carried out by analyzing the drug solutions within same day. The inter-day precision was measured using standard solution over three consecutive days. Reproducibility of the method was determined by performing same analytical procedure at

different laboratories using same experimental design.

Robustness

The robustness of the method was investigated under a variety of conditions including changes of pH of the eluent, flow rate and of buffer composition. The obtained results were compared with that of standard results.

Specificity

Specificity was studied in order to assess unequivocally an analyst in the presence of components that may be expected to be present. Specificity was confirmed by obtaining positive results (by comparison with a known reference material) from samples containing the analyst, coupled with negative results from samples which do not contain the analyst. The parameters like retention time (t_R), resolution (R_S) capacity factor, tailing factor were calculated.

Application

The applicability of the proposed methods were determined by analysis of laboratory synthetic mixture containing 5 mg EM and 500 mg MT with other commonly used excipients in tablet formulation.

RESULT AND DISCUSSION

Method Development

Different columns containing Octyl and Octadecylsilane stationary phases were tried for the separation and resolution. It was found that HYPERSIL column offered more advantages. Individual drug solution was injected into column and elution pattern of all the drugs and resolution parameters were studied. In addition to this, UV spectra of individual drugs were recorded at the wavelength from 200 to 400nm and the response for optimization was compared. The choice of wavelength 260nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.

Method Validation

System Suitability

System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution(R), retention time (RT) were determined. The results are shown in Table 1, it indicates good performance of system.

Table 1: System Performance for MetforminHcl and Sitagliptin phosphate.

Drug substances	Retention time	Symmetry factor T*	No of plates	Resolution factor R
Metformin Hcl	*2.030*	*1.52*	*2443*	-
Sitagliptin phosphate	*2.790*	*1.57*	*4615*	*4.626*

Linearity

Under the experimental conditions described above, linear calibration curves for the two drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the two drugs (y) v/s concentration (x). The linear ranges of Metformin Hcl and Sitagliptin are 20-120µg/ml, 2-8µg/ml respectively showed in (Table 2), (figure 1 a,b & 2)

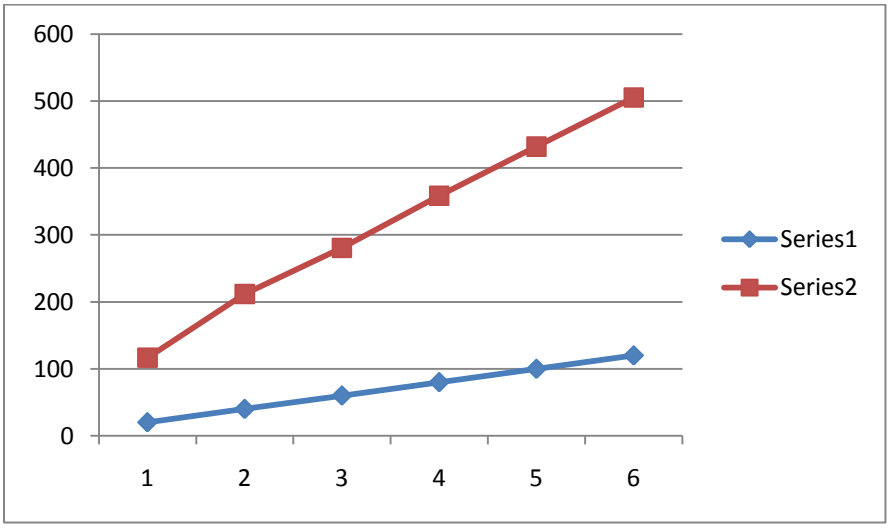

Figure 1 a: Calibrated graph of Metformin hydrochloride shows the linearity of Metformin hydrochloride.

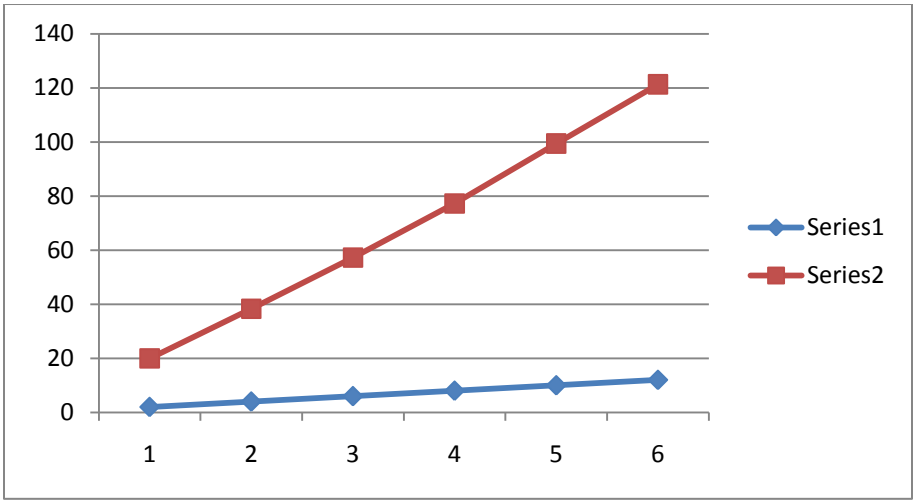

Figure 1 b: Calibrated graph of Sitagliptin phosphate shows the linearity of Sitagliptin phosphate.

Table 2: Linearity – Regression analysis data

Parameters	MetforminHcl	Sitagliptin Phosphate
Correlation coefficien(r)	0.9996	0.9991
Intercept(b)	262.3	-1.1
Slope(m)	3.8	10.05

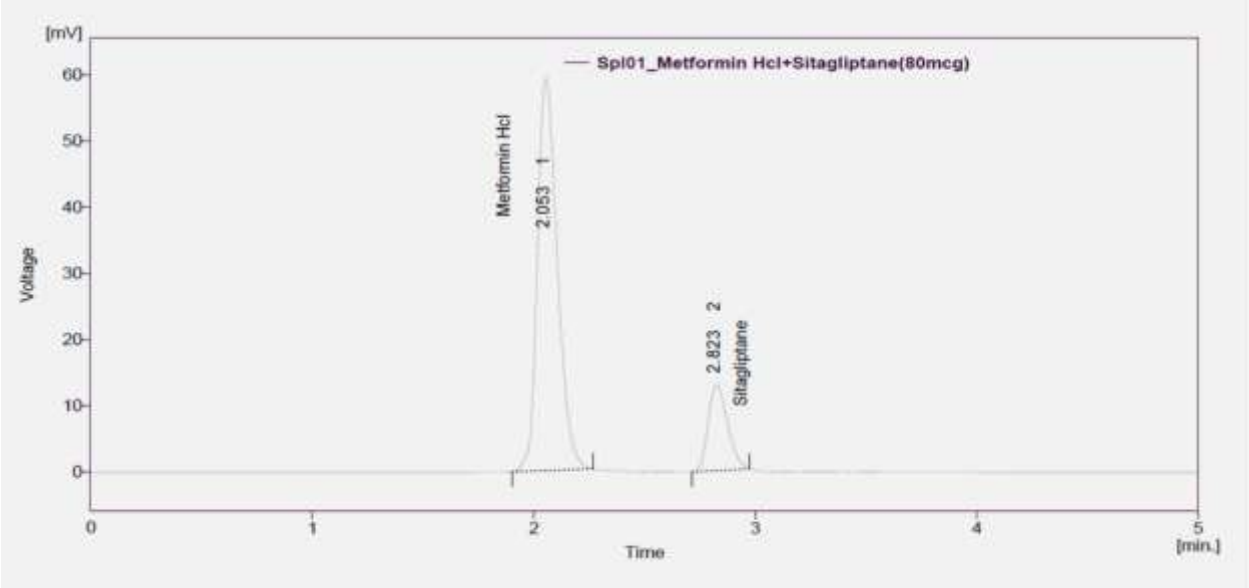

Figure 2: Chromatogram of Metfotmin hydrochloride and Sitagliptin phosphate Assay.

Accuracy

Accuracy of the method was determined by applying the proposed method to synthetic mixture containing known amount of each drug to 80%, 100%, and 120% of the label claim. The accuracy was then calculated as the percentage of analyze recovered by the assay. The results of the recovery analysis are enclosed under (Table 3).

Precision

The assay was carried out of two drugs using proposed method in six replicates. The value of relative standard deviation lie well within the limits (0.09% or Metformin and 0.59% for Sitagliptin), it indicates the sample repeatability of the method enclosed in (Table 4).

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in flow rate by ± 0.1ml/min.

Variation in wavelength by ± 2nm.

Table 3: Accuracy-% Recovery of each analyte

Drug name	conc% of Spiked level	% Recovery	Mean % recovery	Standard deviation	%RSD
Metformine HCL	**80%**	99.24	99.89	0.60119	0.60176
	80%	100.03			
	80%	100.42			
	100%	**99.45**	**99.86**	**1.18924**	**1.1990**
	100%	**98.93**			
	100%	**101.20**			
	120%	**99.62**	**99.53**	**1.56694**	**1.57433**
	120%	**101.05**			
	120%	**97.92**			
Sitagliptin Phosphate	**80%**	**99.32**	**99.28**	**0.03464**	**0.03424**
	80%	**99.28**			
	80%	**99.28**			
	100%	**99.66**	**99.59**	**0.0577**	**0.0579**
	100%	**99.56**			
	100%	**99.56**			
	120%	**99.24**	**99.16**	**0.066**	**0.067**
	120%	**99.12**			
	120%	**99.13**			

Table 4: Method Precision of MetforminHCL and Sitagliptin phosphate

Injection	Peak areas of metformine HCL	Peak areas of Sitagliptin phosphate
1	368.256	80.353
2	369.097	80.152
3	369.357	81.632
4	369.563	79.412
5	370.054	80.530
Mean	369.2654	80.4158
SD	0.664472	0.802081
%RSD	0.179944	0.994717

Stability of Solution

Stock solution of sample and standard contains 80µg/ml Metformin Hcl, 8µg/ml Sitagliptin phosphate. Stock solution stability was checked for 24hrs at room temperature. The drug solution was found to be stable for the specified period.

Method Application

The validated high performance liquid chromatography method was applied to simultaneous determination of Metformin Hcl and Sitagliptin phosphate. Locally available tablet dosage form contains Metformin Hcl 500mg and Sitagliptin phosphate 50mg. 20 tablets were crushed and powdered , weigh powder equivalent to 50mg of Metformin Hcl and transfer into 50ml volumetric flask, add 50ml of mobile phase. It was sonicated at 35^{o}c for 6min to dissolve completely. This solution was further diluted to get a solution having concentration of 80µg/ml Metformin Hcl, 8µg/ml Sitagliptin phosphate. 20µl of this solution was injected into the chromatograph under the specified chromatographic conditions. The analyte peaks were identified by comparisions with those of respective standard for their retention time. The peak areas were used to calculate the drugs. The assay results, expressed as % of the label claim, are in (Table.5). this indicates that the amount of each drug in the product meets the requirements.

Table 5: Method application (Assay).

Peak name	RT	Area	%Area	Tailing Factor	Resolution
Metformin	**2.030**	**358.327**	**82.263**	**1.571**	**-**
Sitagliptin phosphate	**2.790**	**77.258**	**12.681**	**1.455**	**4.626**

CONCLUSION

The proposed HPLC method provides as a fast, accurate and rugged assay with stability indicating potential for these two drugs in tablet or in solution alone. In conclusion, the developed method is strongly recommended for the assay of two drugs in the locally available pharmaceutical dosage form i.e. tablet.

ACKNOWLEDGEMENT

The principal and Head of the Pharmaceutical Analysis Department of Browns college of pharmacy, A.P, for guidance, encouragement and providing laboratory facilities.

REFERENCES

1. Government of India, Ministry of health and family welfare, Indian Pharmacopoeia, Vol- I & II, The Controller of Publication, New Delhi ; 2010: 1657-60.
2. The United States pharmacopoeia, USP 34 NF 29, Vol- III United States pharmacopoeia convention, lnc, Rockville; 2011: 3442.
3. Arayne MS, Najma S, Zuberi MH, Siddiqui FA. Spectrophotometric Quantitation of Metformin in bulk drug & Pharmaceutical formulation using multivariate technique. Indian J Pharm Sci 2009; 71:331-35.

4. Mousumi K, Choudary PK. HPLC method for estimation of Metformin hydrochloride in formulated microspheres & tablet dosage form. Indian J Pharm Sci 2009; 2:318-20.
5. Balasekaran C, PrameelaRani A. Development & Validation of Spectrophotometric method for its determination of DPP-4-inhibitor, Sitagliptin, in its Pharmaceutical preparations. Int J Pharm Pharm Sci 2010; 2:138-42.
6. Tang Y, Li X, Wen N, Sun Xu, Yu M, LI ZG. RP-HPLC determination of Sitagliptin phosphate. Yaowu Fenxi Zazhi, 2009; 29:1370-72.
7. ICH Q2A; Guidelines on validation of analytical procedure; definitions and terminology, Federal Register 1995; 60: 11260.
8. ICH Q2B; Guidelines on validation of analytical procedure; Methodology, Federal register 1996; 60: 27464.