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Method Development and Validation for the Simultaneous Estimation of Metformin and Sitagliptin by RP-HPLC

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ABSTRACT

As there are very few reports on the simultaneous estimation of Metformin and Sitagliptin, a new RP-HPLC method was developed and validated. It was found from the results presented that the proposed method has good sensitivity, precision and accuracy. As chromatographic run time is 4.5 min, it allows the analysis of a large number of samples in a short period of time. The percentage recovery of the combination of Metformin and Sitagliptin was found to be in the range of 98.7%-100.01%. Therefore it is suitable for the routine analysis of Metformin and Sitagliptin in pharmaceutical dosage forms. The present method succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in co-formulated formulation. The results of the study indicate that the proposed RP-HPLC method was simple, rapid, precise and accurate. Statistical analysis proves that this method was reproducible and selective for the combination analysis of Sitagliptin and Metformin. It can therefore be concluded that use of this method can save much time, very economic and that can be with accuracy.

INTRODUCTION

Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture which contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called “retention time”. A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel.

Partition chromatography was the first kind of chromatography that chemists developed. The partition coefficient principle has been applied in paper chromatography, thin layer chromatography, gas phase and liquid-liquid applications. The 1952 Nobel Prize in chemistry was earned by Archer John Porter Martin and Richard Laurence Millington Synge for their development of the technique, which was used for their separation of amino acids. Partition chromatography uses a retained solvent, on the surface or within the grains or fibers of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some additional coulombic and or hydrogen donor interaction with the solid support. Molecules equilibrate (partition) between a liquid stationary phase and the eluent. Known as Hydrophilic Interaction Chromatography (HILIC) in HPLC, this method separates analytes based on polar differences. HILIC most often uses a bonded polar stationary phase and a non-polar, water miscible, mobile phase. Partition HPLC has been used historically on unbonded silica or alumina supports. Each works effectively for separating analytes by relative polar differences, however, HILIC has the advantage of separating acidic, basic and neutral solutes in a single chromatogram.

Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are more non-polar,

while polar molecules elute more readily. An investigator can increase retention time by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RPC is so commonly used that it is often incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

MATERIALS AND METHODS

List of instruments used:

HPLC system: Shimadzu LC10AT- Pump, SPD10A-Detector (Isocratic) VP Series, Software: Spinchrom
Shimadzu LC20AT- Pump, SPD20A- Detector (Gradient), Prominence
Software: Spinchrom
UV-Visible Spectrophotometer (Thermo NICOLET Evolution 100)
Analytical weighing balance (Shimadzu AY220)
Ultra Sonicator (Ultra Sonic Cleaner)
pH meter (Global Digital DPH500)
Hot air oven (Rotek)
Vacuum filter pump,
Filtration kit
Mobile phase reservoir

List of chemicals used:

HPLC grade Acetonitrile (Qualigens)
Methanol (Qualigens)
Orthophosphoric acid (MERCK)
Potassium dihydrogen Phosphate (MERCK)
HPLC Water (Millipore filtered)

Optimized chromatographic conditions:

Equipment Sampler and UV detector	: High performance liquid chromatography equipped with Auto
Column	: Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra)
Flow rate	: 1 mL per min
Wavelength	: 260 nm
Injection volume	: 10 µl
Column oven	: Ambient
Run time	: 10min

Preparation of mobile phase:

500 mL (50%) of the 0.1%orthophosphoric acid and 500 mL of methanol HPLC grade (50%) were mixed thoroughly and degassed in ultrasonic water bath for 5 minutes, filtered through 0.45 µ filter under vacuum filtration.

Standard preparation:

Accurately weigh and transfer 100 mg of Metformin and 10 mg of Sitagliptin working standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 5 minutes and makeup to the final volume with diluents (standard stock).

Sample preparation:

20 tablets were weighed and calculate the average weight of tablets. A weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further, the volume made up with diluent and filtered. From the filtered solution, 0.4 ml was pipetted out into a 10ml volumetric flask and made up to 10 ml with diluent.

RESULTS

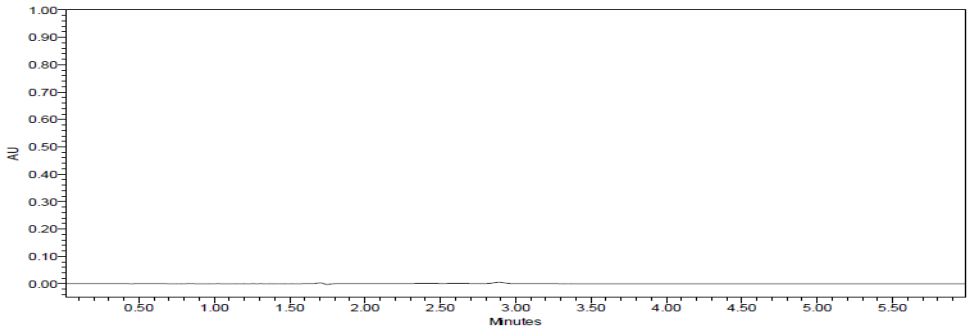
System Suitability and System Precision for Sitagliptin:

S. No.	RT	AREA	USP PLATE COUNT	USP TAILING
1	3.683	219848	4902	1.43
2	3.673	221565	4926	1.44
3	3.675	219812	4915	1.41
4	3.681	221878	4790	1.43
5	3.686	221152	4856	1.43
6	3.691	221421	4905	1.42
Mean	3.6815	220946	4882	1.43

System Suitability and System Precision for Metformin:

S. No.	RT	Area	USP Plate count	USP Tailing
1	1.774	357969	6289	1.48
2	1.760	361586	5360	1.42
3	1.759	363182	5429	1.43
4	1.759	361588	5324	1.43
5	1.766	359015	5459	1.44
6	1.770	359280	5435	1.43
Mean	1.764	360436	5549	1.43

Chromatogram for Blank:

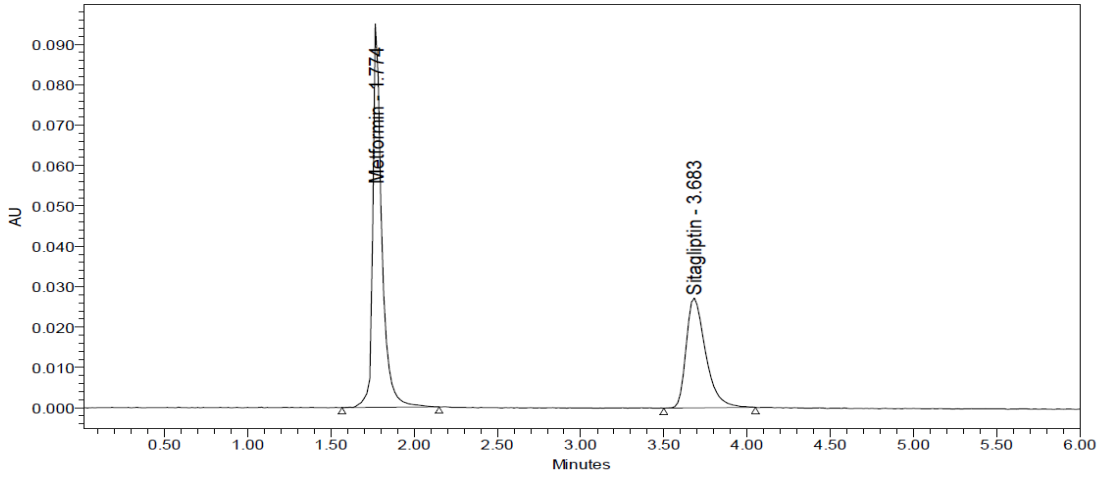


	Peak Name	RT
1	Metformin	1.774
2	Sitagliptin	3.683

Chromatogram for Standard:

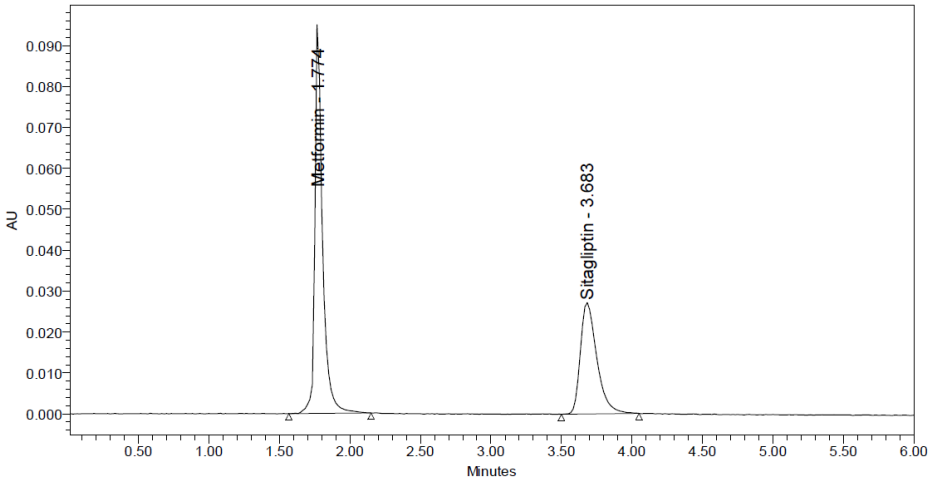
SAMPLE INFORMATION

Sample Name:	Standard	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Metform_sitaglipt_Validation
Vial:	2	Acq. Method Set:	Metformin_Sitagliptin_Assay
Injection #:	1	Processing Method:	Metformin_Sitagliptin
Injection Volume:	20.00 ul	Channel Name:	260.0nm
Run Time:	6.0 Minutes	Proc. Chnl. Descr.:	PDA 260.0 nm
Date Acquired:	5/3/2012 9:37:42 PM IST		
Date Processed:	5/7/2012 12:34:39 PM IST		



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.774	357969	61.95	6289	1.48
2	Sitagliptin	3.683	219848	38.05	4902	1.43

Accuracy:



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.774	357969	61.95	6289	1.48
2	Sitagliptin	3.683	219848	38.05	4902	1.43

Accuracy of Metformin:

Sample ID	Concentration	Percentage Recovery	Mean Percentage Recovery	Standard deviation	Relative standard deviation
1	50%	101.2953	100.373	1.807	1.804
2	50%	101.536			
3	50%	98.287			
4	100%	99.2998	100.263	1.19	1.19
5	100%	101.101			
6	100%	101.564			
7	150%	98.333	98.39	0.081	0.082
8	150%	98.36			
9	150%	99.93			

Accuracy of Sitagliptin:

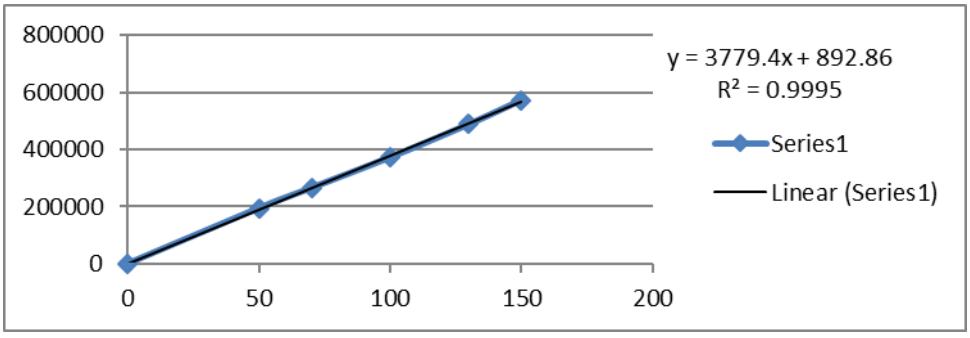
Sample ID	Concentration mcg	Percentage Recovery	Mean Percentage Recovery	Standard deviation	Relative standard deviation
1	50%	98.7905	98.97	0.3159	0.31
2	50%	99.3374			
3	50%	98.7923			
4	100%	101.387	101.74	1.36	1.34
5	100%	100.582			
6	100%	103.24			
7	150%	98.623	98.94508	0.274	0.277
8	150%	99.06			
9	150%	99.15			

Linearity:

Linearity of Metformin:

Concentration of Metformin (ppm)	Peak Area
0	0
50	194715
70	265641
100	372832
130	488901
150	572973
Correlation Coefficient	0.9995

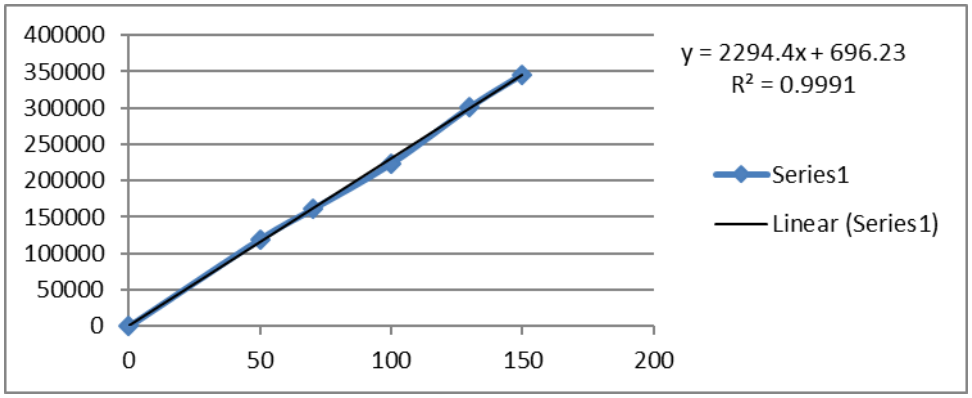
Calibration curve of Metformin:



Linearity of Sitagliptin:

Concentration of Sitagliptin (ppm)	Peak Area
0	0
50	119633
70	160921
100	223536
130	301128
150	346153
Correlation Coefficient	0.9995

Calibration curve of Sitagliptin:



DISCUSSION

From the linearity table it was found that the drug obeys linearity within the concentration range of 50-150 µg/ml for metformin and sitagliptin. From the results shown in accuracy Table, it was found that the percentage recovery values of pure drug were in between 99.8 to 101.9, which indicate that the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering the proposed method. From the results shown in precision Tables, It was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

CONCLUSION

As there are very few reports on the simultaneous estimation of Metformin and Sitagliptin, a new RP-HPLC method was developed and validated. It was found from the results presented that the proposed method has good sensitivity, precision and accuracy. As chromatographic run time is 4.5min, it allows the analysis of a large number of samples in a short period of time. The percentage recovery of the combination of Metformin and Sitagliptin was found to be in the range of 98.7%-100.01%. Therefore it is suitable for the routine analysis of in pharmaceutical dosage forms. The present method succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in co-formulated formulation. The results of the study indicate that the proposed RP-HPLC method was simple, rapid, precise and accurate. Statistical analysis proves that, this method was reproducible and selective for the combination analysis of Sitagliptin and Metformin. It can therefore be concluded that use of this method can save much time, very economic and that can be with accuracy.

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I humbly present this work to the eternal almighty. Indeed my final work is done with the help of primitive persons at heart. So it is my bounded duty to promulgate them individually.

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ABBREVIATIONS

HPLC	High performance Liquid Chromatography
%	Percent
PDA	Photodiode Array
ICH	International Conference for Harmonization
GR	General reagent
C18	Octadecyl
UV	Ultraviolet
ml	Milliliter
Min	Minute
MeOH	Methanol
µl	Micro Liter
µ	Micron
µg	Micro gram
ppm	Parts per million
nm	Nanometer
RSD	Relative Standard Deviation
Fig	Figure

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