Research Article

Method Development and Validation of A Stability-Indicating Reversed-Phase Liquid Chromatographic Method for the Simultaneous Estimation of Metformin, Sitagliptin and Simvastatin in Presence of their Degradation Products

N. C. Kotecha* [1,2], **J. K. Patel** [1,3]
Gujarat Technological University, Ahmedabad, Gujarat, India [1]
University of Colorado, Anschutz medical campus, Denver, USA [2]
Nootan Pharmacy College, Visnagar, Gujarat, India[3]
***Corresponding author's E-mail:** nckotecha@gmail.com

Received: 08-04-2018; **Revised:** 29-04-2018; **Accepted:** 09-05-2018.

ABSTRACT

A reversed-phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of Metformin, Sitagliptin, and Simvastatin in the presence of their degradation products. Analytes were separated on a Hypersil C18, 250x4.6 mm, 5µm column using an isocratic elution mode having mobile phase composition of 50 mM potassium dihydrogen phosphate buffer (pH 3.5): acetonitrile: tri-ethylamine (85:15:0.1 %v/v/v). Analytes were detected at a wavelength of 225 nm. A 20µL fixed-loop injector was used for the injection of the samples with a flow rate of 1.0 mL min−1. The optimized method was validated as per ICH Q2 guidelines. The retention times of Metformin, Sitagliptin, and Simvastatin were 3.70 min, 5.10 and 6.84 min, respectively. The linearity was 25-100 µg/ml for Metformin, 2-8 µg/ml Sitagliptin and 2.5-10 µg/ml Simvastatin. The correlation coefficient for calibration curves of Metformin, Sitagliptin, and Simvastatin was> 0.99. Accuracy was 98-102% for each analyte. Inter and Intra-day precision was calculated < 2 %RSD for each analyte. Limit of detection (LOD) and limit of quantitation (LOQ) were within the limits of ICH-Q2 guidelines. The method was robust with % RSD values < 2% with the deliberate changes in the composition of mobile phase, changes in the pH or change in the flow rate. Significant degradation was observed in the presence of acidic, basic, neutral, oxidative and photolytic stress conditions. The proposed RP-HPLC method is simple, precise, accurate, robust and reproducible and was able to successfully separate and quantify Metformin, Sitagliptin, and Simvastatin in the presence of their degradation products; this implies the stability indicating nature and specificity of the method.

Keywords: Metformin, Sitagliptin, Simvastatin, stress testing, degradation products, stability-indicating method, HPLC.

INTRODUCTION

Type 2 Diabetes mellitus (T2DM) is the most prevalent metabolic disease worldwide. Inadequate management and control of hyperglycemia in patients with T2DM may lead to the risk of developing complications over the long term due to chronic and progressive nature of the disease arising from pathophysiology of beta-cell dysfunction, insulin resistance and increased hepatic glucose output. Patients with T2DM often require a combination of therapeutic agents in order to achieve glycemic control over the long term [1-6].

Dyslipidemia is a major predisposing factor for atherosclerotic cardiovascular disease (CVD) in the general population as well as in diabetic patients. Because of increased CVD risk, most guidelines that address treatment of dyslipidemia in patients with diabetes consider diabetes as a CVD "risk equivalent" and recommend intensive treatment of dyslipidemia for the purpose of CVD prevention. These treatment guidelines provide goals for lipids and glucose levels [1-4].

Fixed-dose combination (FDC) therapies have been shown to improve adherence by reducing costs, pill burden, and the complexity of treatment regimen [8-10]. A treatment approach with a FDC that includes a statin and anti-diabetic medication could be used to improve statin compliance in patients with type 2 diabetes [1]. A combined formulation consisting in a single tablet would potentially offer increased patient convenience and subsequent potential for increased therapeutic compliance. The fixed dose combination of Metformin, Sitagliptin and Simvastatin can be studied for the treatment of adults with inadequately controlled T2DM to improve treatment of dyslipidemia. Merk Inc. started the clinical trial of A Study of the Efficacy and Safety of MK-0431D (a Fixed-dose Combination of Sitagliptin and Simvastatin) for the Treatment of Participants With Type 2 Diabetes Mellitus (T2DM) with inadequate glycemic control on Metformin monotherapy (MK-0431D-266). The purpose of this study is to assess the efficacy and safety of Sitagliptin/Simvastatin fixed-dose combination (FDC) in participants with T2DM who have inadequate glycemic control while on metformin monotherapy [14]. Advantages of simultaneous stability studies are the identification of new impurities in addition to those studied for stability assays of Metformin, Sitagliptin and Simvastatin alone, to understand mutual induction and/or inhibition of rates of degradation and to analyze common impurities of both drugs in combined dosage forms. The method can be successfully applied for the determination of stability during pre-formulation and formulation studies for the development of fixed dose combination of Metformin, Sitagliptin and Simvastatin.

Various ultraviolet spectroscopic and high performance liquid chromatographic assay methods were reported for the estimation of Metformin, Sitagliptin and Simvastatin individually and in combination with other drugs [14-46]. All the above reported methods were based on the estimation of Metformin, Sitagliptin or Simvastatin alone or in combination with other drugs. As there is a requirement for a suitable stability indicating method for simultaneous estimation of Metformin, Sitagliptin and Simvastatin, the present study has been taken up with an objective to develop a stability-indicating RP-HPLC method for the simultaneous estimation of Metformin, Sitagliptin and Simvastatin by stress degradation to reveal possible degradation products in the combined dosage forms.

This is the first report of a simple, precise, accurate, sensitive and reproducible stability indicating method for the simultaneous estimation of Metformin, Sitagliptin and Simvastatin.

MATERIALS AND METHODS

Instrumentation

Analytes were scanned between 200-400 nm using UV-visible spectrophotometer (Shimadzu, model UV-1700). Experiments were carried out using Shimadzu prominence Modular HPLC system with LC 20AT solvent delivery unit, CBM 20A system controller, SIL 20A auto-sampler, CTO 20A column oven and SPD 20 A UV Detector. Data was recorded and evaluated using Spinchrom software as the data integrator. 20µL fixed-loop injector was used for the injection of the samples with the flow rate of 1.0 mL min−1. The pH of the solutions was measured with the pH meter (Mettler Toledo, S20K). Refluxing of the drugs in specific degradation conditions were carried out using a Rotavapor (R-300, Buchi). A Shimadzu ATX-124 analytical balance was used for weighing.

Reagents and Chemicals

The Metformin, Sitagliptin and Simvastatin reference materials were purchased from Mesochem Technology, Inc., Beijing, China. Methanol and Water were used of HPLC grade and purchased from Fisher Scientific, India. Potassium dihydrogen phosphate buffer was purchased from Sigma-Aldrich Company, India.

Selection of wavelength

Standard solution of Metformin, Sitagliptin (10 µg/mL) and Simvastatin (10 µg/mL) were scanned between 200-400 nm using a UV-visible spectrophotometer. Wavelength was selected from the overlay spectra of above solutions.

Chromatographic separation

Analytes were separated on Agilent XDB-C8, 150 x 4.6 mm, 5µm column using an isocratic elution mode having mobile phase composition of 50 mM potassium dihydrogen phosphate buffer (pH 3.5): acetonitrile: tri-ethyl amine (85:15:0.1 %v/v/v). The detection was carried out at the wavelength of 225 nm. Peak area, peak height, retention time and resolution were recorded using Spinchrom software. 20µL fixed-loop injector was used for the injection of the samples with the flow rate of 1.0 mL min−1.

Preparation of standard solutions

50 mg of Metformin, 4 mg of Sitagliptin and 5 mg of Simvastatin were separately weighed and transferred to previously labeled 100 mL volumetric flasks and volume was made up to the mark with methanol to obtain 500 µg/mL of Metformin standard stock solution, 40 µg/mL Sitagliptin standard stock solution and 50 µg/mL of Simvastatin standard stock solution. 1 mL from each of the Metformin, Sitagliptin and Simvastatin stock solutions were transferred into labeled 10 mL volumetric flasks and volume was made up to the mark by mobile phase to obtain a standard solution of mixtures of Metformin (50 µg/mL), Sitagliptin (4 µg/mL) and Simvastatin (5 µg/mL).

Method Validation

System suitability test

System suitability test is an integral part of the chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use [40].

Linearity

The linearity was assessed by analysis of combined standard solution in a range of 25- 75 µg/ml for Metformin, 2- 6 µg/ml Sitagliptin and 2.5- 7.5 µg/ml Simvastatin.

Precision

Results were expressed as percentage relative standard deviation (%RSD) or coefficient of variance.

Repeatability

A standard solution containing 50 µg/ml of Metformin, 4 µg/ml of Sitagliptin and 5 µg/ml of Simvastatin was injected six times, areas of peaks were measured and % RSD was calculated to determine the repeatability of the method.

Intra- day and inter-day precision

A standard solution containing (20, 50, 75 µg/ml) of Metformin; (2, 4, 6 µg/ml) of Sitagliptin and (2.5, 5, 7.5 µg/ml) of Simvastatin were analyzed three times on the same day for the determination of intra-day precision and on three different days for the determination of inter-day precision and % R.S.D was calculated.

Accuracy

Accuracy was calculated at three different levels in terms of % recovery by spiking known amount of standard solution (80%, 100% and 120%) to the solution of a synthetic laboratory mixture of Metformin, Sitagliptin and Simvastatin.

Specificity and selectivity

The specificity of the method was established through the study of resolution factors of the drug peak from the nearest resolving peak and also among all other peaks.

Limit of detection and Limit of quantitation (LOD and LOQ)

The LOD and LOQ were estimated at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

Robustness

Robustness of the method was investigated by varying the chromatographic conditions, such as, changing the flow rate by ± 10% i.e. 0.8 ml/min and 1.2 ml/min; changing the ratio of mobile phase was with ±2 i.e. 50 mM potassium dihydrogen phosphate buffer (pH 3.5): acetonitrile: tri-ethyl amine (83:17:0.1 %v/v/v) and (87:13:0.1 %v/v/v); and changing the pH of the buffer in the mobile phase with ± 0.2% i.e. 3.7 and 3.3. Robustness of the developed method was indicated by the overall % RSD between the data, at each variable condition.

Analysis of marketed formulation

Synthetic laboratory mixture of with 50 mg of Metformin, 4 mg of Sitagliptin and 5 mg of Simvastatin were weighed individually and spiked with 1 mg Hydroxy propyl cellulose (E463) and 1 mg Micro Crystalline Cellulose (E460 (i)) as tablet excipients into a 100 ml volumetric flask. The analytes were extracted with 5 ml methanol by sonication in the ultra-sonicator bath and then the volume was made up to the mark with mobile phase. The solution was filtered through Whatman filter paper no. 42. One mL from this solution was transferred to 25 ml volumetric flask and volume was made up to the mark with mobile phase to obtain the concentration of 50 µg/ml for Metformin, 4 µg/ml Sitagliptin and 5 µg/ml for Simvastatin. Samples were analyzed using the developed assay. The areas of resulting peaks were measured at 225 nm.

Stress degradation studies

Acid hydrolysis

Forced degradation in acidic condition was performed by adding 1 ml of standard solution of mixtures of Metformin (500 µg/ml), Sitagliptin (40 µg/ml) and Simvastatin (50 µg/ml) to 10 ml each of methanol and 0.1 M hydrochloric acid and refluxing the mixture at 70°C for 4 hours (n=3). The solution was then allowed to reach at room temperature, neutralized to pH 7.0 by the addition of 0.1 M sodium hydroxide, and diluted to 100 ml with the mobile phase so as to get a final concentration of 50 µg/ml of Metformin, 4 µg/ml of Sitagliptin and 5 µg/ml of Simvastatin.

Alkaline hydrolysis

Alkali-induced, forced degradation was performed by adding 1 ml of a standard solution of a mixture of Metformin (500 µg/ml), Sitagliptin (40 µg/ml) and Simvastatin (50 µg/ml) to 10 ml each of methanol and 0.1 M sodium hydroxide and refluxing the mixture at 70°C for 2 hours. The solution was then allowed to reach at room temperature, neutralized to pH 7.0 by the addition of 0.1 M hydrochloric acid, and diluted to 100 ml with the mobile phase to get a final concentration of 50 µg/ml of Metformin, 4 µg/ml of Sitagliptin and 5 µg/ml of Simvastatin.

Oxidative degradation

To evaluate the effect of oxidizing conditions, 1 ml of the standard solution of a mixture of Metformin (500 µg/ml), Sitagliptin (40 µg/ml) and Simvastatin (50 µg/ml) was added to 2 ml of 3% hydrogen peroxide solution and the mixture was refluxed at 70°C for 2 hours. The solution was then allowed to reach room temperature and diluted to 100 ml with the mobile phase to get a final concentration of 50 µg/ml of Metformin, 4 µg/ml of Sitagliptin and 5 µg/ml of Simvastatin.

Thermal degradation

To evaluate the effect of temperature, 1 ml of a standard solution of a mixture of Metformin (500 µg/ml), Sitagliptin (40 µg/ml) and Simvastatin (50 µg/ml) was stored at 105°C in a hot air oven for 1.5 hours. The solution was then allowed to reach room temperature and diluted to 100 ml with the mobile phase to get a final concentration of 50 µg/ml of Metformin, 4 µg/ml of Sitagliptin and 5 µg/ml of Simvastatin.

Photolytic degradation

To study the effect of UV light, 1 ml of a standard solution of a mixture of Metformin (500 µg/ml), Sitagliptin (40 µg/ml) and Simvastatin (50 µg/ml) was exposed to short and long wavelength UV light (254 nm and 366 nm, respectively) for 24 hours, and then dissolved in 10 ml of methanol. The volume was made up by the mobile phase in a 50 ml volumetric flask and then 1 ml of stock solution was further diluted with the mobile phase to give a solution of final concentration equivalent to 50 µg/ml of Metformin, 4 µg/ml of Sitagliptin and 5 µg/ml of Simvastatin.

Synthetic laboratory mixture was also treated with described acidic, alkaline, oxidative, thermal and photolytic degradation conditions. Twenty microliters of the resulting solutions were injected into the HPLC system and the chromatograms were recorded.

RESULTS AND DISCUSSION

Method development

As Metformin, Sitagliptin and Simvastatin both showed absorbance response at a wavelength of 225 nm, it was selected as a wavelength of detection. Figure 2 represents the overlying UV spectra of Metformin, Sitagliptin and Simvastatin.

For the initial trials during method development, reverse phase chromatography was chosen because of its simple and convenient use in terms of efficiency, stability and reproducibility. Analytes were separated on Agilent XDB-C8, 150 x 4.6 mm, 5µm column using an isocratic elution mode having mobile phase composition of 50 mM potassium dihydrogen phosphate buffer (pH 3.5): acetonitrile: tri-ethyl amine (83:17:0.1 %v/v/v). Analytes were detected at 225 nm. 20µL fixed-loop injector was used for the injection of the samples with the flow rate of 1.0 mL min−1. Retention times were 3.70 min, 5.10 and 6.84 min for Metformin, Sitagliptin and Simvastatin respectively, as shown in Figure 3.

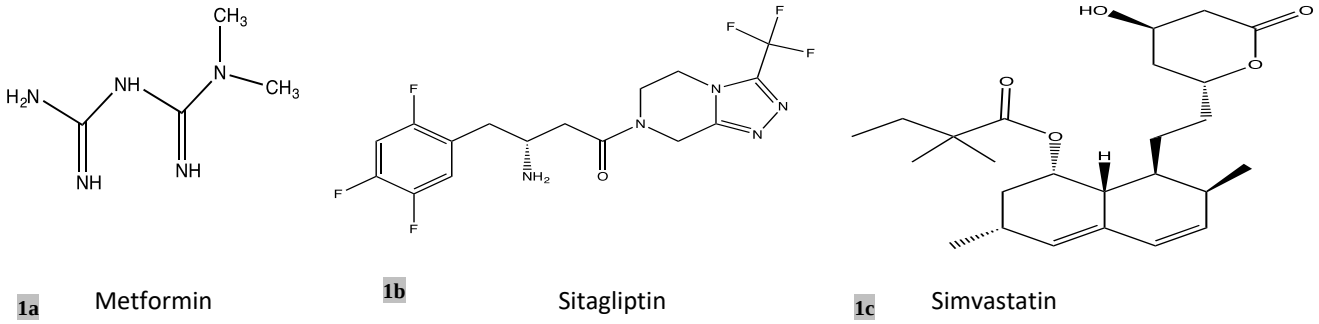

Figure 1: Chemical structures of Metformin, Sitagliptin and Simvastatin

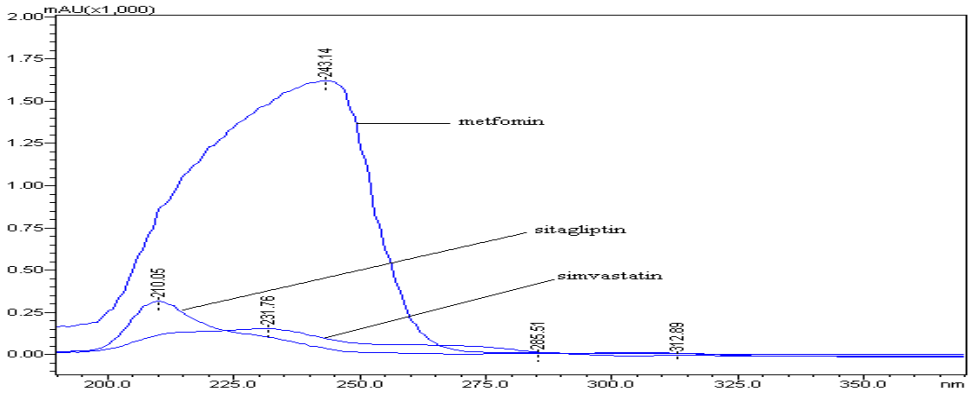

Figure 2: Overlay UV Spectrum of Metformin, Sitagliptin and Simvastatin showing selection of wavelength detection.

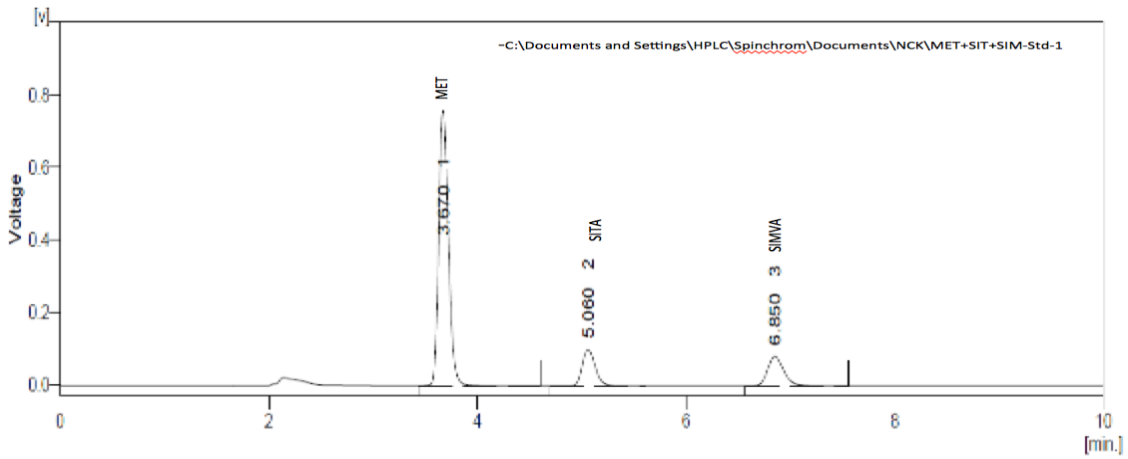

Figure 3: Chromatogram of Metformin, Sitagliptin and Simvastatin in 50 mM potassium dihydrogen phosphate buffer (pH 3.5): acetonitrile: tri-ethyl amine (85:15:0.1 %v/v/v) with flow rate-1.0 ml/min

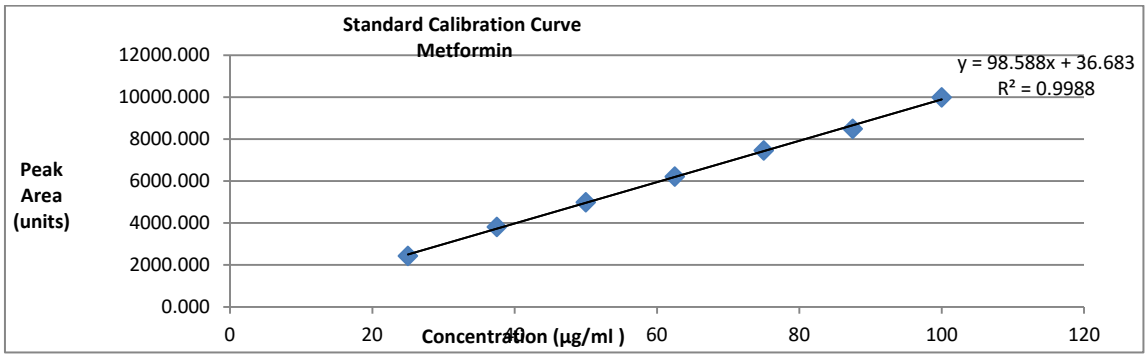

Figure 4: Standard Calibration curve of Metformin (25-75 µg/ml)

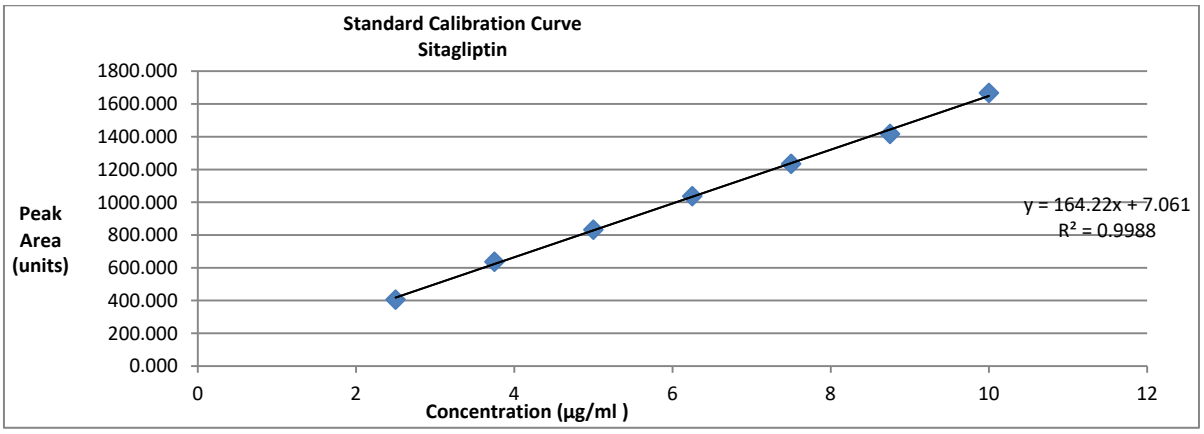

Figure 5: Standard Calibration curve of Simvastatin (2-8 µg/ml)

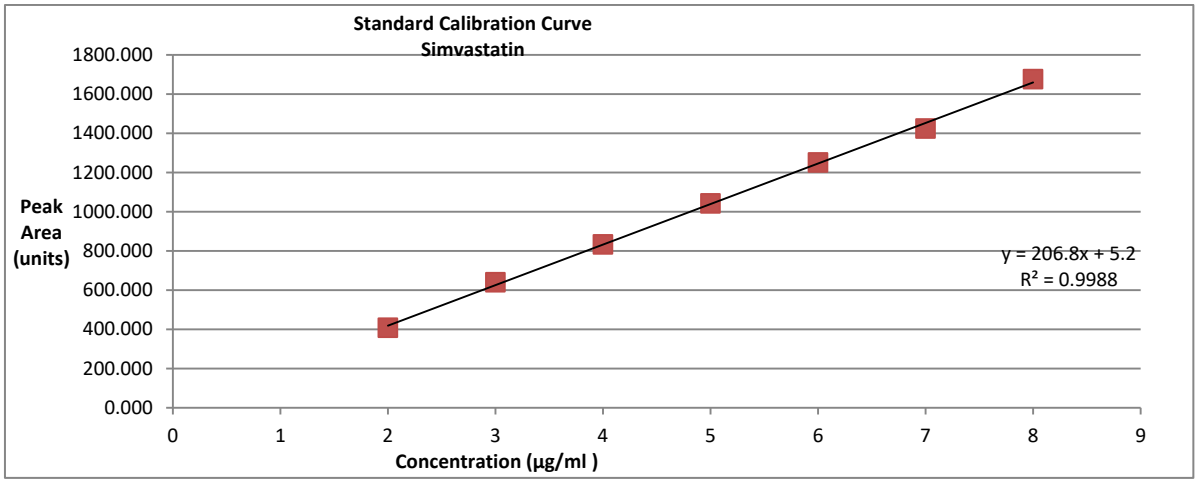

Figure 6: Standard Calibration curve of Sitagliptin (2.5-7.5 µg/ml)

Method validation

Table 1: System suitability parameters for Metformin, Sitagliptin and Simvastatin

System Suitability Parameters	Metformin	Sitagliptin	Simvastatin
Theoretical plates per column (N)	7461	9358	7594
Symmetry factor/Tailing factor	1.133	1.359	1.131
Resolution	6.912		6.945

Table 2: Results from regression analysis for Metformin, Sitagliptin and Simvastatin

Description	Metformin	Sitagliptin	Simvastatin
Linearity and range	25- 75 µg/ml	2- 6 µg/ml	2.5- 7.5 µg/ml
Regression co-efficient	0.998	0.998	0.998
Slope (m)	98.58	164.22	206.7
Intercept (c)	36.68	7.06	5.2

Table 3: Repeatability data for Metformin, Sitagliptin and Simvastatin

Metformin				Sitagliptin				Simvastatin			
Conc. (µg/ml)	Peak Area	Mean ± S.D (n=6)	% R.S.D	Conc. (µg/ml)	Peak Area	Mean ± S.D (n=6)	% R.S.D	Conc. (µg/ml)	Peak Area	Mean ± S.D (n=6)	% R.S.D
10	4969.0	5011.8±39.7	0.79	10	829.5	833.8 ±12.4	1.5	4	833.3	835.5 ±13.1	1.57
	5018.8				837.8				841.6		
	4957.1				810.3				833.2		
	5028.6				839.4				811.7		
	5043.6				841.9				845.8		
	5053.6				843.6				847.4		

Table 4: Intra-day and Inter-day precision for Metformin, Sitagliptin and Simvastatin

Metformin			Sitagliptin			Simvastatin		
Conc. (µg/ml)	Mean ± S.D (n=6)	% R.S.D	Conc. (µg/ml)	Mean ± S.D (n=6)	% R.S.D	Conc. (µg/ml)	Mean ± S.D (n=6)	% R.S.D
Intra-day precision								
25	2495.5 ± 2.8	0.11	2.5	416.5 ± 0.8	0.19	2	418.3± 1.3	0.31
50	4960.8± 14.3	0.28	5.0	827.1± 4.5	0.54	4	825.2± 14.0	1.70
75	7492.2± 26.8	0.35	7.5	1249.3± 4.6	0.37	6	1254.0 ±3.2	0.25
Inter-day precision								
25	2489.3 ± 27.5	1.10	2.5	414.5 ± 6.9	1.66	2	416.9 ± 5.8	1.40
50	4954.2± 34.7	0.70	5.0	826.2± 5.4	0.66	4	827.5± 6.4	0.78
75	7429.8± 52.8	0.71	7.5	1239.1 ± 11.0	0.88	6	1244.6 ±8.8	0.70

Table 5.1: Accuracy in terms of % recovery for Metformin

Conc. Level (%)	Sample amount (µg/ml)	Amount of Standard Added (µg/ml)	Metformin		
			Amount Recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
80 %	25	20	20.1	100.9	100.9 ± 0.5
	25	20	20.0	100.4	
	25	20	20.3	101.5	
100 %	25	25	25.1	100.4	100.4 ± 0.6
	25	25	25.2	101.1	
	25	25	24.9	99.7	
120 %	25	30	29.9	99.6	99.4 ± 0.3
	25	30	29.7	99.0	
	25	30	29.8	99.5	

Table 5.2: Accuracy in terms of % recovery for Sitagliptin

Conc. Level (%)	Sample amount (µg/ml)	Amount of Standard Added (µg/ml)	Sitagliptin		
			Amount Recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
80 %	2.5	2	2.0	100.3	100.9 ± 0.6
	2.5	2	2.0	100.9	
	2.5	2	2.0	101.5	
100 %	2.5	2.5	2.5	100.5	100.6 ± 0.5
	2.5	2.5	2.5	101.2	
	2.5	2.5	2.5	100.1	
120 %	2.5	3	2.9	99.2	99.2 ± 0.4
	2.5	3	2.9	98.7	
	2.5	3	2.9	99.6	

Table 5.3: Accuracy in terms of % recovery for Simvastatin

Conc. Level (%)	Sample amount (µg/ml)	Amount of Standard Added (µg/ml)	Simvastatin		
			Amount Recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
80 %	20	16	15.8	98.8	100.0 ± 1.0
	20	16	16.1	101.0	
	20	16	16.0	100.1	
100 %	20	20	20.0	100.4	100.3 ± 1.4
	20	20	20.3	101.7	
	20	20	19.7	98.9	
120 %	20	24	23.9	99.9	99.3 ± 0.6
	20	24	23.6	98.5	
	20	24	23.8	99.5	

Table 6: Analysis of synthetic mixture by developed method

Synthetic laboratory mixture	Analyte		
Weight(mg) of synthetic laboratory mixture	Metformin (50 mg)	Sitagliptin (4 mg)	Simvastatin (5 mg)
Assay Mean ± S. D. (n=6)	98.5±0.25	101.0±0.30	98.1±0.24

Table 7: Percent degradation of Metformin with retention time of the degradation products

Sr. No.	Conditions	Retention time of Metformin/ degradation products (min)	% degradation of Metfromin (n=5)	% degradation of Metformin in synthetic mixture (n=5)
1	Untreated stock solution (10µg/ml)	3.78	-	-
2	Acid hydrolysis	4.51, 7.96	14.106	16.928
3	Alkali hydrolysis	2.53,3.35,5.12	13.634	13.023
4	Oxidative degradation	3.03,5.35	12.355	12.086
5	Thermal degradation	2.64, 4.66	13.822	11.491
6	Photolytic degradation	2.44, 4.31	13.568	15.419

Table 8: Percent degradation of Sitagliptin with retention time of the degradation products

Sr. No.	Conditions	Retention time of Sitagliptin / degradation products (min)	% degradation of Sitagliptin (n=5)	% degradation of Sitagliptin in synthetic mixture (n=5)
1	Untreated stock solution (10µg/ml)	5.06	-	-
2	Acid hydrolysis	4.12,7.29	14.312	17.077
3	Alkali hydrolysis	2.83, 5.00	12.224	13.080
4	Oxidative degradation	2.90, 5.14	12.725	12.389
5	Thermal degradation	2.90, 5.12	12.554	13.590
6	Photolytic degradation	2.82, 4.97	13.858	14.027

Table 9: Percent degradation of Simvastatin with retention time of the degradation products

Sr. No.	Conditions	Retention time of Simvastatin / degradation products (min)	% degradation of Simvastatin (n=5)	% degradation of Simvastatin in synthetic mixture (n=5)
1	Untreated stock solution (10µg/ml)	6.85	-	-
2	Acid hydrolysis	4.12,7.29	19.441	16.977
3	Alkali hydrolysis	2.83, 5.00	18.953	15.863
4	Oxidative degradation	2.90, 5.14	19.857	15.381
5	Thermal degradation	2.90, 5.12	12.543	13.220
6	Photolytic degradation	2.82, 4.97	11.560	14.875

The method was validated as per ICH guidelines [11] with respect to parameters defining linearity, precision, accuracy, specificity, and robustness.

The number of theoretical plates, peak tailing and resolution factor were determined to define system suitability parameters for Metformin, Sitagliptin and Simvastatin. The results for system suitability data are listed in Table 1. Linearity and range were assessed by analysis of combined standard solution in the range of 25-75 µg/ml for Metformin, 2- 6 µg/ml Sitagliptin and 2.5-7.5 µg/ml Simvastatin. Standard calibration curve for Metformin, Sitagliptin and Simvastatin are represented as Figure 4, 5 and 6 respectively. The data for regression analysis is listed in Table 2. A standard solution containing 50 µg/ml of Metformin, 4 µg/ml of Sitagliptin and 5 µg/ml of Simvastatin respectively was injected six times and areas of peaks were measured to determine the repeatability of the method. % R.S.D. value for the determination of repeatability is represented in Table 3.

A standard solution containing (20, 50, 75 µg/ml) of Metformin; (2, 4, 6 µg/ml) of Sitagliptin and (2.5, 5, 7.5 µg/ml) of Simvastatin were analyzed three times on the same day for the determination of intra-day precision and on three different days for the determination of inter-day precision. % R.S.D values for intra-day and inter-day precision are represented in Table 4. The accuracy of the method was confirmed by recovery study from the synthetic laboratory mixture at three levels of standard addition. The results are shown in Table 5.1, 5.2 and 5.3 for Metformin, Sitagliptin and Simvastatin, respectively. Percentage recovery was in the range of 99.4 - 100.994% for Metformin, 99.2 - 100.9 Sitagliptin and 99.3 - 100.4 % for Simvastatin. LOD was 3.40 µg/ml, 0.22 µg/ml and 0.16 µg/ml for Metformin, Sitagliptin and Simvastatin respectively whereas LOQ was 10.6 µg/ml, 0.68 µg/ml and 0.48 µg/ml for Metformin, Sitagliptin and Simvastatin respectively. The method was robust with % RSD values <2% with the deliberate changes in the composition of mobile phase, changes in the pH or change in the flow rate. Applicability of the proposed method was evaluated by analyzing a synthetic laboratory mixture and the results are shown in Table 6. The assay results were 99.0 % and 99.8 % of Metformin, Sitagliptin and Simvastatin respectively in synthetic laboratory mixture.

Establishment of stability indicating method for assessment of degradation behavior

The stressed samples were assayed using developed RP-HPLC method. Following degradation behavior was observed under different stress conditions for the high-performance liquid chromatography studies on the combination of Metformin, Sitagliptin and Simvastatin [Table 7-9].

The representative degradation chromatograms are shown in Figure 3-7. Significant degradation was observed in the presence of acidic, basic, neutral oxidative and photolytic stress conditions for Metformin, Sitagliptin and Simvastatin respectively (n=5). Percentage Degradation for the standard drug was 14%, 13%, 12%, 13% and 13% for Metformin; 14%, 12%, 12%, 12% and 13% Sitagliptin and 19%, 18%, 19%, 12% and 11% for Simvastatin in the presence of acidic, basic, thermal, oxidative and photolytic degradation respectively. Percentage Degradation for the Metformin in synthetic laboratory mixture was 16%, 13%, 12%, 11% and 15% in the presence of acidic, basic, thermal, oxidative and photolytic degradation respectively. Percentage Degradation for the Sitagliptin in synthetic laboratory mixture was 17%, 13%, 12%, 13% and 14% in the presence of acidic, basic, thermal, oxidative and photolytic degradation respectively. Percentage degradation for Simvastatin in synthetic laboratory mixture was 16%, 15%, 15%, 13% and 14% in the presence of acidic, basic, thermal, oxidative and photolytic degradation respectively. The percent degradation was calculated by the formula: % degradation = (Average peak area of untreated stock solution – average peak area of stock solution under specific degradation condition)/(average peak area of untreated stock solution) x 100)

CONCLUSION

The overall demand for the fixed dose combination anti-diabetic and anti-hyperlipidemic drug development has been growing in the pharmaceutical market. This increases the need for the development of cost effective and high throughput assays. Proposed reversed phase high performance liquid chromatographic method was able to successfully separate and quantify Metformin, Sitagliptin and Simvastatin simultaneously in the presence of their degradation products. This implies the stability indicating nature and specificity of the method. The developed validated stability indicating RP-HPLC method is simple, precise, accurate, robust and reproducible resolving all the degradation products from the analytes of interest. The proposed method can be applied for the quantitative determination of Metformin, Sitagliptin and Simvastatin in generic drug development industries. The developed method is validated as per ICH guidelines and can be successfully applied in quality control divisions of pharmaceutical industries and therapeutic drug monitoring for clinical trials. The method can also be used for the pre-formulation and formulations development studies in pharmaceutical research laboratories.

Acknowledgments: The authors wish to thank Dr. Rajshree Mashru and Dr. Jolly Parikh for providing their valuable scientific inputs.

REFERENCES

1. Steinberg H, Anderson MS, Musliner T, Hanson ME, & Engel SS, Management of dyslipidemia and hyperglycemia with a fixed-dose combination of sitagliptin and simvastatin, Vascular Health and Risk Management, 9, 2013, 273–282.

2. Brunzell JD, Davidson M, Furberg CD, Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. J Am Coll Cardiol, 51, 2008, 1512–1524.
3. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report, Circulation, 106, 2002, 3143–3421.
4. Genest J, McPherson R, Frohlich J, Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult – 2009 recommendations, Can J Cardiol, 25, 2009, 567–579.
5. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S) Lancet, 344, 1994, 1383–1389 [No authors listed]
6. Cannon CP, Braunwald E, McCabe CH, Intensive versus moderate lipid lowering with statins after acute coronary syndromes, N Engl J Med, 350, 2004, 1495–1504.
7. LaRosa JC, Grundy SM, Waters DD, et al Intensive lipid lowering with atorvastatin in patients with stable coronary disease, N Engl J Med, 352, 2005, 1425–1435.
8. Cheong C, Barner JC, Lawson KA, Johnsrud MT, Patient adherence and reimbursement amount for antidiabetic fixed-dose combination products compared with dual therapy among Texas Medicaid recipients, Clin Ther, 30, 2008, 1893–1907.
9. Pan F, Chernew ME, Fendrick AM, Impact of fixed-dose combination drugs on adherence to prescription medications, J Gen Intern Med, 23, 2008, 611–614.
10. Thayer S, Arondekar B, Harley C, Darkow TE, Adherence to a fixed-dose combination of rosiglitazone/glimepiride in subjects switching from monotherapy or dual therapy with a thiazolidinedione and/or a sulfonylurea, Ann Pharmacother, 44, 2010, 791–799.
11. Drucker DJ, Nauck MA, The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes, Lancet, 368, 2006, 1696–1705.
12. Herman GA, Stein PP, Thornberry NA, Wagner JA, Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes: focus on Sitagliptin, Clin Pharmacol Ther, 81, 2007, 761–767.
13. Zocor (simvastatin) tablets [package insert] Whitehouse Station, NJ: Merck and Co, Inc, 2011.
14. https://clinicaltrials.gov/ct2/show/NCT01678820
15. Drug profile for Sitagliptin, http://www.drugbank.ca/drugs/DB01261 (Accessed on 18/04/2017)
16. Drug profile for Metformin, http://www.drugbank.ca/drugs/DB00331 (Accessed on 18/04/2017)
17. Drug profile for Simvastatin http://www.drugbank.ca/drugs/DB00641 (Accessed on 20/8/04/2017)
18. Shyamala M, Mohideen S, Satyanarayana T, Sures P, Validated RP-HPLC For Simultaneous Estimation Of Sitagliptin Phosphate And Metformin Hydrochloride In Tablet Dosage Form, American J Pharma Res, 1(2), 2011, 93-101.
19. Kashid AM, Dhange AA, Gavande VT, Miniyar PB, RP-HPLC Method Development and Validation For Sitagliptin In Human Plasma, American J Pharma Res, 2(5), 2012, 805-811.
20. Loni AB, Ghante MR, Sawant SD, Simultaneous UV Spectrophotometric Method For Estimation Of Sitagliptin Phosphate And Metformin Hydrochloride In Bulk And Tablet Dosage Form, Der Pharma Chemica, 4(3), 2012, 854-859.
21. Jeyabalan G, Nyola N, Simultaneous Estimation Of Sitagliptin Phosphate Monohydrate And Metformin Hydrochloride In Bulk And Pharmaceutical Formulation By RP-HPLC, J Pharm Educ Res, 3(2), 2012, 24-28.
22. Phaneemdra D, Venktesh V, Ramarao N, Simultaneous Estimation Of Simvastatin And Sitagliptin By Using Different Analytical Methods, Int J In Adv In Pharma Anal, 2(1), 2012, 19-23.
23. Lavanya R, Yunoos MD, Development And Validation Of RP-HPLC Method For The Estimation Of Sitagliptin Phosphate In Bulk And Its Tablet Dosage Form J Adv Pharm Edu & Res, 3(4), 2013, 475-479.
24. Salim M, Enany N, Belal F, Walash M, Simultaneous Determination Of Sitagliptin And Metformin In Pharmaceutical Preparations By Capillary Zone Electrophoresis And Its Application To Human Plasma Analysis, Anal Chem, 7, 2012, 31-46.
25. Warghade SV, Devlekar ND, Patil RY, Barge VU, Validated HPTLC Method For Simultaneous Estimation Of Metformin Hydrochloride And Sitagliptin Phosphate In Bulk Drug And Pharmaceutical Formulation, J Pharm Anal, 5(3), 2014, 2340-47.
26. Kumar SA, Debnath M, Rao LN, Development Of Stability Indicating RP- HPLC Method For Simultaneous Estimation Of Metformin Hydrochloride And Sitagliptin Phosphate Monohydrate In Bulk As Well As In Pharmaceutical Formulation, Der Pharmacia Sinica, 4(4), 2013, 47-61.
27. Malleshwar CS, Surnarayana MV, Mukanti K, Simultaneous Determination Of Sitagliptin Phosphate Monohydrate And Metformin Hydrochloride In Tablets By A Validated UPLC Method, Sci Pharm, 80(1), 2012 Mar, 139–152.
28. Gananadhamu S, Laxmikanth V, Shantikumar S, Rapid LC-ESI-MS/MS Method For The Simultaneous Determination Of Sitagliptin And Pioglitazone In Rat Plasma And Its Application To Pharmacokinetic Study, Sci Res, 3(12), 2012, 849-858
29. Ramalingam P, Bhaskar VU, Reddy YP, Stability Indicating RP-HPLC Method For The Simultaneous Determination Of Sitagliptin And Simvastatin In Tablets, Ind J Pharma Sci, 76(5), 2014, 407-414
30. Sharma S, Manocha N, Estimation Of Sitagliptin Phosphate And Simvastatin And Validation Of The Proposed Method In A Combined Marketed Tablet Dosage Form By Simultaneous

Equation Method, Int J of Current Pharma Res, 4(3), 2012, 144-147

31. Ceren Y, Nuran O, Alper G, Simultaneous Determination Of Rosiglitazone And Metformin In Plasma By Gradient Liquid Chromatography With UV Detection, Talanta, 72(4), 2007, 1416-22.
32. Mousumi K, Choudhury PK, HPLC Method For Estimation Of Metformin Hydrochloride In Formulated Microspheres And Tablet Dosage Form, Indian J Pharm Sci, 71(3), 2009, 318-20.
33. Vasudevan M, Ravi J, Ravisankar S, Suresh B, Ion-pair liquid chromatography technique for the estimation of metformin in its multicomponent dosage forms, J Pharmaceut Biomed, 25(1), 2001, 77-84.
34. Gandhimathi M, Anandakumar K, Cheriyan A, Ravi T, Simultaneous Estimation Of Metformin And Gliclazide In Tablets Using Reverse Phase High Performance Liquid Chromatography, Indian J Pharm Sci, 65(5), 2003, 530-531.
35. Lad NR, Bhoir SI, BhoirI C, Sundaresan M, Concurrent Assay Of Metformin And Glimepiride In Tablets Using RP-HPLC With Wavelength Programming, Indian J Pharm Sci, 65(6), 2003, 650-3
36. Sahoo PK, Sharma R, Chaturvedi SC, Simultaneous Estimation Of Metformin Hydrochloride And Pioglitazone Hydrochloride By RP-HPLC Method From Combined Tablet Dosage Form, Indian J Pharm Sci, 70 (3), 2008, 383-386.
37. Ceren Y, Nuran O, Method Development And Validation For The Simultaneous Determination Of Rosiglitazone And Metformin In Pharmaceutical Preparations By Capillary Zone Electrophoresis, Anal Chim Acta, 549(1-2), 2005, 88-95.
38. Mane VB, Babar S, Kulkarni N, Development Of UV Spectrophotometric Method For The Simultaneous Estimation Of Simvastatin And Ezetimibe In Tablet Dosage Form By Simultaneous Equation And Absorbance Ratio Method, Int J of Pharma Res, 3(3), 2011, 1459-1466
39. Dey S, De A, Mandal S, Pradhan P, Development And Validation Of RP-HPLC Method For The Estimation Of Simvastatin In Bulk And Pharmaceutical Dosage Form, Indo Ame J of Pharma Res, 2013, 7376-7384
40. Praveen Kumar S N, Bhadre Gowda D G, Vathsala Deepu C, Mantelingu K and Rangappa K S Simultaneous Estimation Of Statins Like Pravastatin, Atorvastatin And Simvastatin In Bulk And Pharmaceutical Dosage Form By Means Of High-Performance Liquid Chromatography J of Chem and Pharma res 5(5), 2013, 359-364
41. Nilesh Jain, Ruchi Jain, Hemant Swami, RP-HPLC Method for Simultaneous Estimation of Simvastatin and Ezetimibe in Bulk Drug and its Combined Dosage Form, Asian J Research Chem 1(1), July-Sept 2008,
42. Chaudhari BG, Patel NM, Shah PB, Stability Indicating Reversed Phase Liquid Chromatographic method for simultaneous determination of Simvastatin and Ezetimibe from their combination drug products, J AOAC Int , 90(5), 2007, 1242-1249
43. Rahman MU, Praveen G,Nayola NK, Simultaneous Estimation Of Simvastatin And Ezetimibe In Pharmaceutical Tablet Dosage Forms By RP-HPLC, Int J of Pharma, 2010, 56-62
44. Jain N, Jain R, Swami H, Pandey S, Spectrophotometric Method For Simultaneous Estimation Of Simvastatin And Ezetimibe In Bulk Drug And Its Combined dosage Form, Int J of Pharma and Pharma Sci, 1(1), 2009, 170-175
45. Singala V, Bhaskar R, Simultaneous Estimation Of Simvastatin And Metformin Hydrochloride In Bulk And Solid Dosage Forms, Rasayan J Chem, 3(3), 2010, 507-513
46. Jyothi AP,Tejaswi K,Parimala SS, Spectrophotometric Estimation of Simvastatin in Bulk and Tablet Dosage Form, Int J of Innovative Pharma Res, 4(1), 2013, 284-287

Source of Support: Nil, **Conflict of Interest:** None.