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A NEW RP HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SIMVASTATIN AND SITAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple and selective LC method is described for the determination of Sitagliptin and Simvastatin in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumesof Mixed buffer, 40 volumes methanol and 20 volumes of Acetonitrile with detection of 241 nm. Linearity was observed in the range 60-140µg /ml for Sitagliptin(r^2 =0.997) and 61-155µg /ml for Simvastatin (r^2 =0.997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. From the above experimental results and parameters, it was concluded that, this newly developed method for the simultaneous estimation Sitagliptin and Simvastatin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

Key Words: Sitagliptin, Simvastatin, tablet dosage forms, LC method

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INTRODUCTION
Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities ,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department . The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions (1,2).

Sitagliptin works to competitively inhibit the enzymedipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretinsGLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and

suppress the release of glucagon by the alpha cells of the pancreas. This drives blood glucose levels towards normal. As the blood glucose level approaches normal, the amounts of insulin released and glucagon suppressed diminishes, thus tending to prevent an "overshoot" and subsequent low blood sugar (hypoglycemia) which is seen with some other oral hypoglycemic agents.Simvastatin is a prodrug in which the 6-membered lactone ring of simvastatin is hydrolyzed *in vivo* to generate the beta,delta-dihydroxy acid, an active metabolite structurally similar to HMG-CoA (hydroxymethylglutaryl CoA). Once hydrolyzed, simvastatin competes with HMG-CoA for HMG-CoA reductase, a hepatic microsomal enzyme, which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis.[2] Simvastatin acts primarily in the liver, where decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increases hepatic uptake of LDL. Simvastatin also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL (3,4).Aim is to develop new RP-HPLC method for the simultaneous estimation of Simvastatin and Sitagliptinin pharmaceutical dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Sitagliptin

10 mg of Sitagliptinwas weighed and transferred in to 10ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Simvastatin

10mg of Simvastatin was weighed in to 10ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 98 mg of Sitagliptinand 102 mg of Simvastatin in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.From above stock solution 98 µg/ml of Sitagliptinand 102 µg/ml of Simvastatin is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

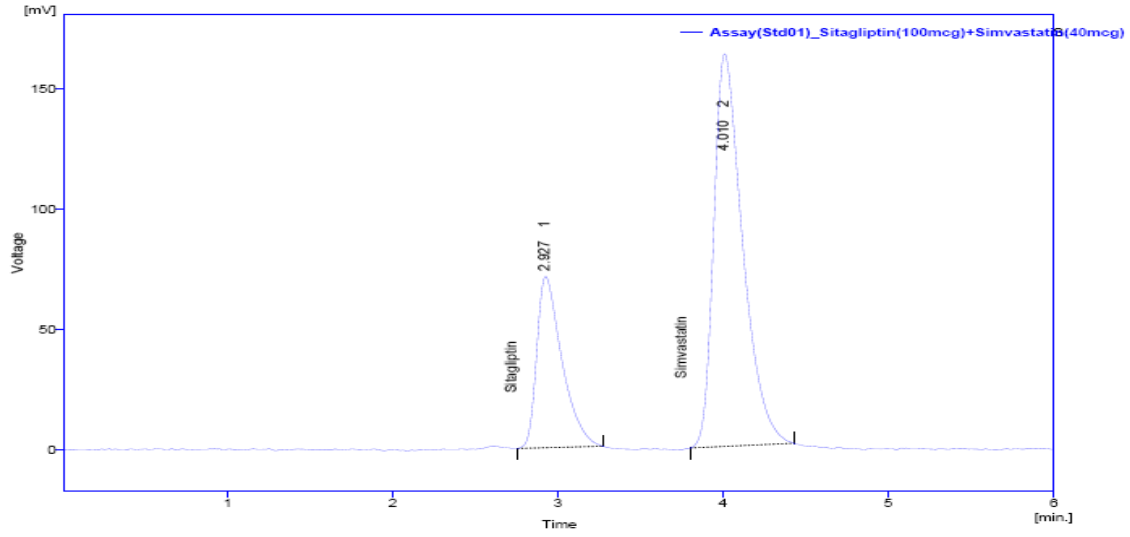
Tablet sample

5tablets (each tablet containsSimvastatin 102mgSitagliptin-98mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Simvastatin and Sitagliptin(µg/ml) were prepared bydissolving weight equivalent to 102 mg of Simvastatinand98 mg of Sitagliptinand dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 102µg/ml of Simvastatin and 98µg/ml of Sitagliptinwas made by adding 1 ml of stock solution to 10 ml of mobile phase. The amount of Simvastatin and Sitagliptinpresent in the formulation by using the formula given below (5-7)

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank.The absorption curve shows characteristic absorption maxima at 230 nm for Sitagliptin, 228nm for Simvastatin and 241 nm for the combination.The amount of Sitagliptinand Simvastatin present in the taken dosage form was found to be 100.17% and 100.55% respectively (fig-1).



Result Table (Uncal - Assay(Std01)_Sitagliptin(100mcg)+Simvastatin(40mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.927	753.879	71.201	27.56	30.4	0.16
2	4.010	1981.613	163.180	72.44	69.6	0.19
	Total	2735.492	234.381	100.00	100.0	

Column Performance Table (From 50% - Assay(Std01)_Sitagliptin(100mcg)+Simvastatin(40mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Effi [t.p./m]	Resolution [-]
1	2.927	0.163	2.161	0.00	1779	17787	-
2	4.010	0.187	1.973	0.00	2557	25566	3.643

Fig-1 Chromatogram of Assay standard preparation

The % RSD for the retention times and peak area of sitagliptin and simvastatin were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table 1 and 2).

Table-1Results for system suitability of sitagliptin

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor	Resolution
1	3.773	295.884	4667	1.111	3.247
2	3.733	290.743	4813	1.143	3.306
3	3.733	292.910	4813	1.176	3.540
4	3.770	293.024	4908	1.206	3.531
5	3.733	290.900	4813	1.176	3.247
6	3.748	292.692	4667	1.111	3.306
Mean	0.0049	55.704	-	-	-
SD	**0.14**	**0.64**	-	-	-
%RSD	3.773	295.884	-	-	-

Table-2 Results for system suitability of Simvastatin

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.680	8815.579	2994	1.596	3.247
2	4.837	8708.391	2058	1.627	3.306
3	4.683	8510.447	2436	1.907	3.540
4	4.670	8553.080	2422	1.952	3.531
5	4.680	8815.579	2994	1.596	3.247
6	4.690	8708.391	2058	1.627	3.306
Mean	4.707	8685.245	-	-	-
SD	0.064	128.893	-	-	-
%RSD	**1.36**	**1.48**	-	-	-

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of sitagliptin and simvastatin is 0.997 and 0.997. The relationship between the concentration of sitagliptin and simvastatin and area of sitagliptin and simvastatin is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Fig-2 and 3).

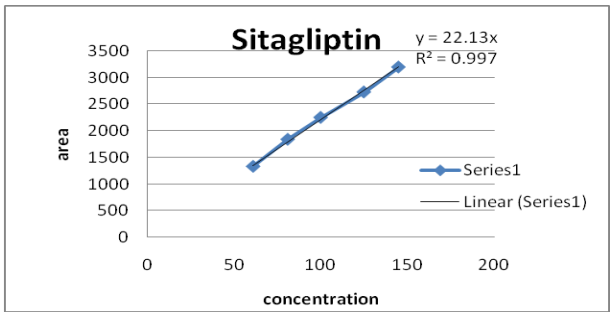


Fig-2 Linearity graph of sitagliptin

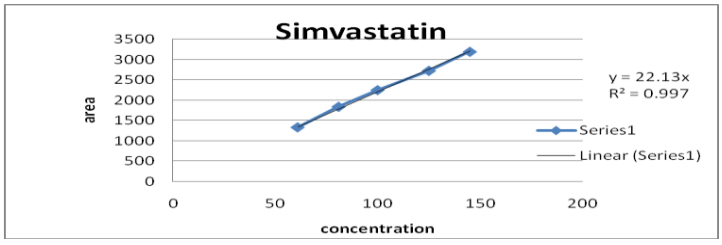


Fig-3 Linearity graph of simvastatin

The percentage mean recovery of sitagliptin and simvastatin is 99.60% and 98.08 % respectively. From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged (Table-3).

Table-3 Results for Ruggedness

SITAGLIPTIN	%Assay	SIMVASTATIN	%Assay
Analyst 01	99.33	**Analyst 01**	99.69
Analyst 02	99.33	**Analyst 02**	99.69

CONCLUSION

From the above experimental results and parameters, it was concluded that, this newly developed method for the simultaneous estimation Sitagliptin and Simvastatin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

REFERENCES

1. Ghazala K, Dinesh S, Agrawal YP, Neetu S, Avnish J, Gupta AK. Simultaneous estimation of metformin and sitagliptin in tablet dosage form. Asian J Biochem Pham Res. 2011;2:223–9.
2. Jain P, Chaudhary A, Desai B, Patel S, Patel S, Shimpi H. Development and validation of first order derivative UV Spectrophotometric method for determination of Sitagliptin in bulk and in Formulation. Int J Drug Dev Res. 2011;3:194–9.
3. Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: Results from two randomized, double-blind, placebo-controlled studies with single oral doses. ClinPharmacolTher. 2005;78:675–88.
4. N. BujjiBabu, R.Srinivasulu and R.Ramesh Raju Simultaneous analysis of RPHPLC reported Method Development and Validation of Enoxaparin sodium and Sitaglipitin drugs in Pharmaceutical dosage form. Journal of Pharmacy Research,2011, Vol.4(11):pp 4029-4031
5. ICH, Text on Validation of Analytical Procedures, ICH – Q2A, International Conference on Harmonisation, IFPMA, Geneva, 1995; 2-3: A–1 to A–3.
6. ICH, Validation of Analytical Procedures Methodology, ICH – Q2B, International Conference on Harmonisation, 1996; p1-3.
7. ICH Guidelines, Q2 (R1) Validation of Analytical Procedures Text and Methodology, 2005; p1-6.