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Development and validation of stabilty indicating RP-HPLC method for simultaneous estimation of metformin and Alogliptin in bulk and tablet dosage form

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Abstract
A new method was established for simultaneous estimation of Metformin and Alogliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Metformin and Alogliptin by using Thermosil C18 (4.0×125 mm) 5.0µm column (4.6×50mm) 3.7µ, flow rate was 0.7 ml/min, mobile phase ratio was Methanol: Sodium acetate buffer (70: 30 % v/v), detection wave length was 252nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Metformin and Alogliptin was found to be 101.03% and 99.27% respectively. The system suitability parameters for Metformin and Alogliptin such as theoretical plates and tailing factor were found to be 1540.37, 1.26 and 4348.8, 1.16, the resolution was found to be 2.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Metformin and Alogliptin was found in concentration range of 50-250ppm and 5ppm-25ppm and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.47%, %RSD for repeatability was 0.8and 0.82, % RSD for intermediate precision analyst 1 was 0.44 and intermediate precision analyst 2 was 0.19 respectively. The precision study was precision, robustness and repeatability. LOD value was 3.17 and 0.0172 and LOQ value was 5.80 and 0.212 respectively.

Keywords: Thermosil c18, metformin and Alogliptin, RP-HPLC, methanol

Introduction

Analytical chemistry[1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

Quality control (**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements

backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants: The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies: The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays: The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

Fundamental and applied research: The chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.

Analytical techniques: There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various *analytical techniques*. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic, molecular spectrometry* and *chromatography*, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. The most important atomic and molecular spectrometric techniques and their principal applications are listed in Table.No.2.

Chromatographic techniques provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called hyphenation, provides a powerful means of separating and identifying unknown compounds.

Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. When used alone, metformin does not cause hypoglycemia; however, it may potentiate the hypoglycemic effects of sulfonylureas and insulin. Its main side effects are dyspepsia, nausea and diarrhea. Alogliptin is a selective, orally-bioavailable inhibitor of enzymatic activity of dipeptidyl peptidase-4 (DPP-4). Chemically, Alogliptin is prepared as a benzoate salt and exists predominantly as the R-enantiomer (>99%). It undergoes little or no chiral conversion *in vivo* to the (S)-enantiomer. FDA approved January 25, 2013.

Chemicals and standards used

Water, Methanol, Acetonitrile, Ortho phosphoric acid, KH2PO4, K2HPO4, 0. 22µ Nylon filter, 0.45µ filter paper, Metformin and Alogliptin.

Method development for the simultaneous estimation of Metformin and Alogliptin by using RP-HPLC.

1. Selection of mobile phase
2. Selection of detection wavelength
3. Selection of column
4. Selection of solvent delivery system
5. Selection of flow rate
6. Selection of column temperature
7. Selection of diluent
8. Selection of test concentration and injection volume

Trial-5 (optimised method)

Chromatographic conditions

Column	:	Thermosil C18 (4.0×125 mm) 5.0µm
Mobile phase ratio	:	Methanol: Sodium acetate buffer (70: 30 % v/v)
Detection wavelength	:	252 nm
Flow rate	:	0.7 ml/min
Injection volume	:	10µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	8min
Retention time	:	2.566 & 3.417 mins

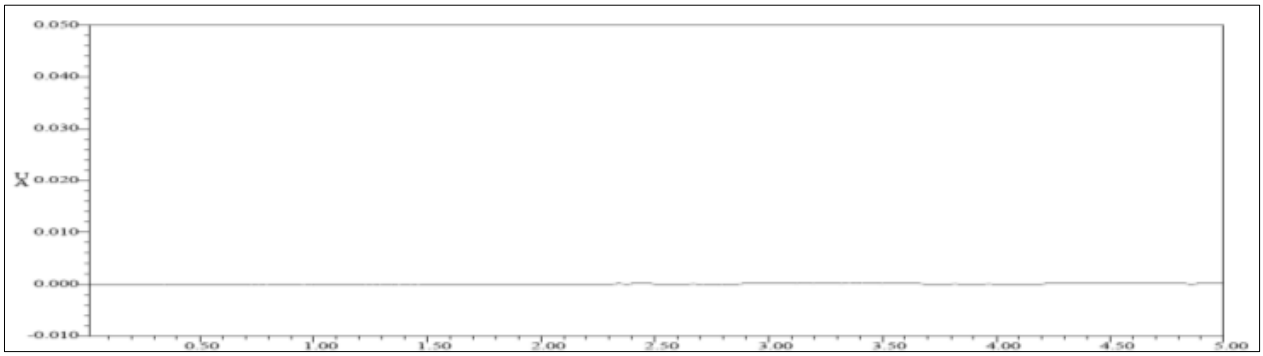


Fig 1: Chromatogram showing blank preparation (mobile phase)

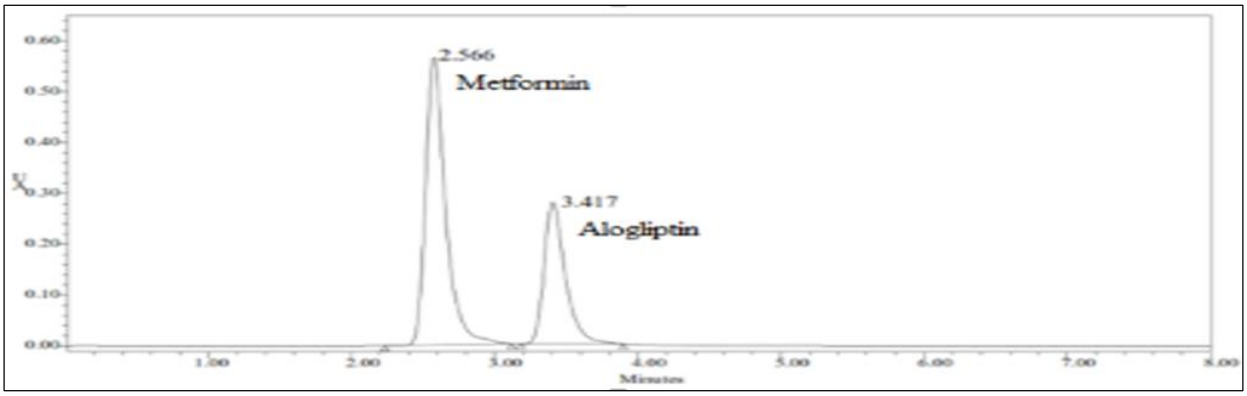


Table 1: Detailsof Trial-5

S. No	Peakname	RT	Area	Height	USP Plate count	USP tailing	USP Resolution
1	Metformin	2.566	78844525	3758466	2421	1.12	
2	Alogliptin	3.417	58446638	283374	2531	1.34	1.01

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Validation Report
System Suitability

Table 2: Results for system suitability of Metformin

Injection	RT (Min)	Peak area	USP Plate Count	Tailing Factor
1	2.567	124452	1564.32	1.24
2	2.568	124857	1534.54	1.21
3	2.569	121804	1523.37	1.31
4	2.570	128383	1523.74	1.24
5	2.571	123154	1560.38	1.31
6	2.572	125344	1535.87	1.25
Mean		126635	-	-
SD		521.0	-	-
%RSD		0.5	-	-

Table 3: Results for system suitability of Alogliptin

1	RT (Min)	Peak Area	USP Plate Count	Tailing Factor
1	3.418	434308	4415.31	1.16
2	3.419	436747	4332.43	1.16
3	3.420	436752	4322.54	1.16
4	3.421	498950	4314.17	1.16
5	3.422	458626	4321.21	1.16
6	3.423	445285	4387.14	1.17
Mean		44531.2	-	-
SD		1237.3	-	-
%RSD		0.2	-	-

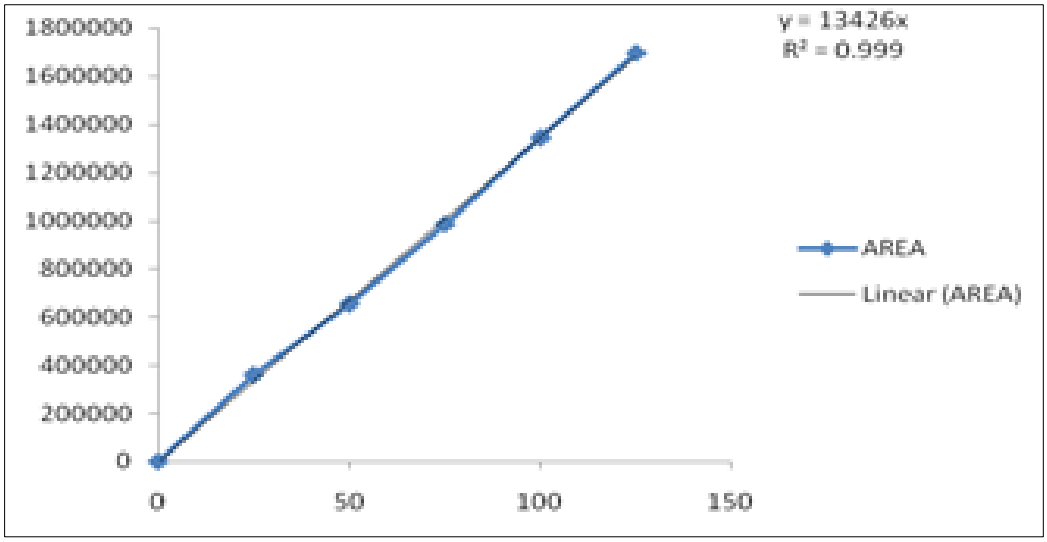
Specificit

Table 4: Results for specificity of Alogliptin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Metformin	2.566	1324531	168042	2123.7	1.5
2	Metformin	3.417	1355112	166102	2116.0	1.5
3	Alogliptin	2.577	326615	45452	2732.2	1.5
4	Alogliptin	3.408	316268	47754	2293.1	1.5

The specificity test was performed for Metformin and Alogliptin. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity



Metformin $r^2 = 0.999$

Fig 3: Showing calibration graph for Metformin

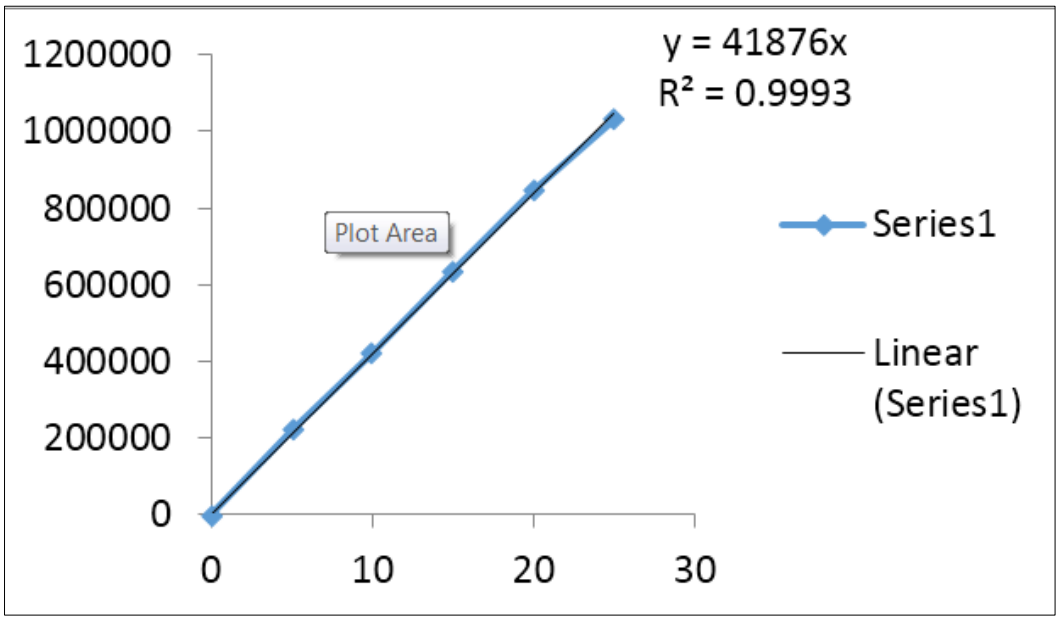


Fig 4: Showing calibration graph for Alogliptin

Accuracy

Table 5: Showing accuracy results for Metformin

% Concentron (at specification level)	Average Area	Amont added (mg)	Amont found (mg)	% Recovery	Mean recovry
50%	2630409	5	4.96	99.91%	99.56%
100%	5277055	10	9.98	99.18%	
150%	7514836	15	14.60	99.60%	

Table 6: Showing accuracy results for Alogliptin

% Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.43	99.53%	99.47%
100%	2777487	1.0	0.93	99.38%	
150%	4151234	1.5	0.95	99.52%	

The accuracy study was performed for % recovery of Metformin and Alogliptin. The % recovery was found to be 99.56% and 99.47% respectively (NLT 98% and NMT 102%)

Precision

Repeatability

Table 7: Showing% RSD results for Metformin

Peak Name: Metformin				
	Peak Name	**RT**	**Area**	**Height (µV) 1**
1.	Metformin	2.755	5223559	541538.3
2.	Metformin	2.632	5208511	485548.5
3.	Metformin	2.687	5323569	574440.4
4.	Metformin	2.612	5259147	557413.5
5.	Metformin	2.616	5273463	565020.1
Mean			5257650	
Std. Dev.			45206.4	
% RSD			0.86	

Table 8: Showing % RSD results for Alogliptin

Peak Name: Alogliptin				
	Peak Name	**RT**	**Area**	**Height (µV) 1**
1.	Alogliptin	3.616	2742453	238643.4
2.	Alogliptin	3.460	2762750	271543.5
3.	Alogliptin	3.634	2797670	281711.6
4.	Alogliptin	3.446	2793578	274499.8
5.	Alogliptin	3.437	2778483	276713.0
Mean			2774987	
Std. Dev.			22806.9	
% RSD			0.89	

Intermediate precision/Ruggedness

Table 9: Showing results for intermediate precision of Metformin

Peak Name: Metformin				
	Peak Name	**RT**	**Area**	**Height (µV) 1**
1.	Metformin	2.688	5698542	539568.1
2.	Metformin	2.633	5682534	536985.4
3.	Metformin	2.613	5695846	539584.1
4.	Metformin	2.617	5689452	534569.8
5.	Metformin	2.756	5636591	534985.5
Mean			5600593	
Std. Dev.			203577.3	
% RSD			0.44	

Table 10: Showing results for intermediate precision of Alogliptin

Peak Name: Alogliptin				
	Peak Name	**RT**	**Area**	**Height (µV) 1**
1.	Alogliptin	3.635	2624315	231325.6
2.	Alogliptin	3.461	2623598	231315.4
3.	Alogliptin	3.447	2623541	231250.1
4.	Alogliptin	3.438	2624987	231342.6
5.	Alogliptin	3.617	2635698	231765.2
Mean			2626428	
Std. Dev.			5215.78	
% RSD			0.19	

The intermediate precision was performed for % RSD of Metformin and Alogliptin was found to be 0.19 and 0.44 respectively (NMT 2).

Detection limit

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Metformin	373625.50	581075863	3.17
Alogliptin	5772.40	476579210	0.0172

The LOD was performed for Metformin and Alogliptin was found to be 3.17and 0.0172 respectively

Quantitation limit

Table 11: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Metformin	372727.80	574265980	5.80
Alogliptin	5761.30	478828490	0.212

Table 12: Showing system suitability results for Metformin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	1	4668	1.3
3	1.2	5216	1.4

Table 13: Showing system suitability results for Alogliptin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	1	6089	1.2
3	1.2	6998	1. 3

Table 14: Showing system suitability results for Metformin

S. No	Change in organi composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	*Actual	4668	1.3
3	5 % more	6387	1.4

Table 15: Showing system suitability results for Alogliptin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	*Actual	6089	1.2
3	5 % more	4817	1.2

Assay

The assay study was performed for the Metformin and Alogliptin. Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in Fig and results are tabulated in Table. The retention time of Metformin and Alogliptin was found to be 2.566 mins and 3.417 mins respectively. The system suitability parameters for Metformin and Alogliptin such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089, 1.2. Resolution was 6.0 the % purity Metformin and Alogliptin in pharmaceutical dosage form was found to be 99.24 and 101.2% respectively.

	Metformin			Alogliptin		
	Standard Area	**Sample Area**	**Assay**	**Standard Area**	**Sample Area**	**Assay**
Injection-1	1333112	1355413	101.67	462181	463759	100.34
Injection-2	1355521	1357013	100.11	465519	463128	99.48
Injection-3	1356115	1356103	99.99	463458	463443	99.99
Average Assay (% purity)			101.03%			99.23%

Observation: the amount of metformin and Alogliptin was found to be 101.03% and 99.23% respectively.

Stability Studies

Table 16: Results of Stress degradation studies

Stress Condition	Sample-1 (Metformin)			Sample-2 (Alogliptin)		
	Area	**% Assay**	**% Degradation**	**Area**	**%Assay**	**% Degradation**
Acidic	13072	90.1	8.7	39575	91.4	8.3
Alkaline	124364	91.0	12.6	348779	81.7	12.4
Photolytic	113659	86.2	13.7	358292	87.4	12.2
Thermal	104374	97.3	11.4	352331	86.3	11.3
Oxidative	105834	94.3	11.2	392422	95.1	11.2

Conclusion

A new method was established for simultaneous estimation of Metformin and Alogliptin by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Metformin and Alogliptin by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoric acid), detection wavelength was 252nm. The instrument used was WATERS HPLC Auto Sampler,
Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Metformin and Alogliptin was found to be 101.27% and 99.97% respectively. The system suitability parameters for Metformin and Alogliptin such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Metformin and Alogliptin was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Metformin and Alogliptin in API and Pharmaceutical dosage form

References

1. Raja *et al.* Determination of metformin in pharmaceutical preparations using potentiometry, spectrofluorimetry and UV–visible spectrophotometry. 1999; 378(1-3):299-311.
2. Satya Sri G *et al.* [Indo American journal of pharmaceutical research]
3. Praveen Kumar *et al.* [International Bulletin of Drug Research. 2013; 3(5):58-68.
4. Maria-Cristina Ranetti *et al.* Farmacia. 2009; 57:6.
5. Ramzia El-Bagary I, Ehab Elkady F, Bassam Ayoub M. Spectroflourometric and Spectrophotometric Methods for the Determination of Sitagliptin in Binary Mixture with Metformin and Ternary Mixture with Metformin and Sitagliptin Alkaline Degradation Product. Sci. 2011; 7(1):62-69.
6. Douglas A Skoog, F James Holler Stanley R. Crouch. Instrumental analysis, India edition, 2007, 13-14.
7. Gurdeep R, Chatwal Sham K, Anand. Instrumental Methods of Chemical Analysis (Analytical Chemistry), 2.566-2.567.
8. Ahuja S Dong MW. Handbook of Pharmaceutical Analysis by HPLC. 1st edition, Academic Press Publisher.UK, 2005.
9. Satinder Ahuja Neil Jespersen. Modern Instrumental Analysis 47 (Comprehensive Analytical Chemistry), 47, 7-8.
10. Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors, New Delhi, 6th edition, 1986, 1-15.
11. Douglas A, Skoog F, James Holler, Timothy A, Nieman. Principles of instrumental analysis, Saunders Golden Sun burst Series, Philadelphia, 2ndedition, 1980, 725-760.
12. David Watson G. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists, Harcourt Publishers Limited, 2nd Edition, 1999, 221-232, 267-311.
13. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Edition, 1997, 1-56, 234-289, 685-712.
14. Beckett AH, JB Stenlake. Practical Pharmaceutical Chemistry, 4th edition. C.B.S. Publications, 53-62.
15. Remingtonn's The Science and Practise of Pharmacy, 20th Edition, 2000.