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Validated HPTLC method for simultaneous estimation of sitagliptin phosphate and simvastatin in tablet dosage form

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ABSTRACT

A novel, simple, sensitive, rapid, accurate and suitable High performance thin layer chromatographic method was developed for the determination of Simvastatin (SIM) and Sitagliptin phosphate (SITA) in bulk and tablet dosage form. A TLC – spectrodensitometric method was developed by the separation of SIM and SIT on silica gel 60_F 254 using a mobile phase mixture of Toluene, Methanol and Acetic acid in a ratio of 5:4:1 v/v/v as a developing system, followed by spectro densitometric measurement of the bands at 255 nm. The R_f values for both the drugs were found to be 0.5241 for SITA and 0.7865 for SIM respectively. Calibration curve was linear over the concentration range of 100-500 ng/ml for Sitagliptin phosphate and 40 – 200 ng/ml for Simvastatin. The suggested method was validated in compliance with the ICH guidelines parameters like Linearity, specificity, precision, accuracy, robustness and ruggedness. The percentage RSD values were found to be 0.22182 for SIM and 0.424151 for SITA respectively. The percentage purity of SIM were found to be 99.78 ± 0.632712 and for SITA 99.9830 ± 0. 175357. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

Key words HPTLC, Methanol, Chromatogram, Water

INTRODUCTION

Diabetes mellitus is difficult to control with a single oral hypoglycaemic agent and the rate of mono therapy failure is high. Hence combination therapy with complementary classes of drugs that act on different aspects of glycaemia control would be expected to be an effective strategy for the control of diabetes. SIM (Fig.1) is chemically 2,2-Dimethylbutanoic acid(1S,3R,7S,8S,8aR)1,2,3,7,8,8ahexahydro-3,7-dimethyl-8-[2-[(2R,4R)tetrahydrohydroxy-6oxo 2H pyran-2yl]ethyl]1-napthalenyl ester used as a HMG - CoA reductase inhibitors. SIM is official in Indian Pharmacopoeia and SITA is official in USP.

SITA 7-[(3R)-3-amino-1-oxo-4-(2,4,5trifluorophenyl)butyl]-5,6,7,8tetrahydro-(trifluoromethyl)-1,2,4-triazolo[4,3-a] pyrazine phosphate used as an oral anti hyperglycemic of the di peptidyl peptidase-4 (DPP-4) inhibitor class. This enzyme-inhibiting drug is used either alone or in combination with other oral anti hyperglycemic agents (such as metformin or thiazolidinedione) for treatment of diabetes mellitus type 2. The benefit of this drug is its lower side-effects (e.g., less hypoglycemia, less weight gain) in the control of blood glucose values. Validated assays have been reported for each drug individually.

A survey of the literature revealed that the methods reported for the determination of SIT are UV spectrophotometry (Safaa m Riad, 2012; Jain Pritam, *et.al.*, 2011;Sheetal Sharma *et.al,* 2012), RP-HPLC Method. (T. Raja and A. Lakshmana rao et.al., 2012; Hitesh P. Inamdar *et.al.*,2012; HPTLC Method B.Stephen Rathinaraj et.al., 2010),Stability indicating UV Spectrophotometric Method Parag Pathade et al., 2010). The methods reported for the determination of SIM are UV spectrophotometry (A Sunitha et al., 2010; RP-HPLC Method. Mujeeb Ur

Rahman et al., 2010; Jayapal Reddy Samaa.C, Rama et al.,2010 ;Nagaraju P. *et al.*, 2009). HPTLC Method T. Raja, A. Lakshmana Rao 2010.)

Moreover the literature survey revealed that so far, no method has been reported for estimation of SIM and SITA in combined dosage form by HPTLC. Therefore the present research work aims to develop a simple, sensitive, accurate and reproducible method for simultaneous estimation of SITA and SIM in combined dosage form by HPTLC method.

Fig-1 Chemical structure of Simvastatin

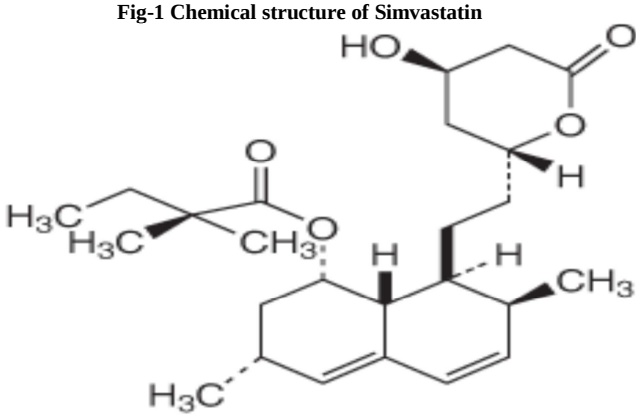
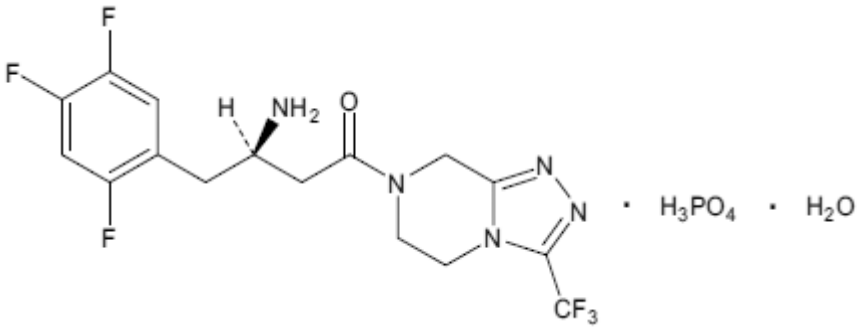


Fig-2 Chemical structure of Sitagliptin Phosphate



MATERIALS ND METHODS

2.1 *Apparatus and software*

The TLC – spectrodensitometric system: Camag TLC Scanner operated with WINCATS software, Camag micro syringe, TLC aluminum sheet (20 x 20cm) precoated with silica gel $60F_{254}$ were used.

2.2 *Chemicals and reagents*

2.2.1 *Pure sample*

The pure drug of SITA and SIM were obtained as a gift sample from Alkem laboratories, Hyderabad. All the apparatus and instruments were calibrated and validated as per calibration and validation protocol specified before starting the experimental work.

2.2.2 *Market sample*

Juvisync tablets containing 40 mg of SIM, 100 mg of SIT was procured from a local Pharmacy, Chennai.

2.2.3 *Solvents*

HPLC grade of Methanol, Acetonitrile, supplied from Sri Rajendra chemicals, Pondicherry. Analytical grade of Glacial acetic acid, Ethyl acetate, Chloroform, Toluene, Diethyl amine, Benzene and distilled water were used.

HPTLC, the separation of the components of a mixture is based on the principle of adsorption. The HPTLC differ from the TLC in the size of silica gel used as the stationary phase and automated sampling application and detection. In the present study a twin trough chamber and silica 60 F 254 were used.

2.3 ***Selection of Stationary Phase***

The resolution of SITA and SIM was achieved using TLC plate made up of silica gel G60 F 254 coated on an Aluminium support (E.Merck).The size of the silica gel particle was 2 µm and thickness of sorbent layer was 0.2 mm. The plates were supplied in 20 × 10 cm size which was cut in to appropriate sizes for method development.

2.3.*1* ***Selection of Mobile Phase***

The mobile phase system was chosen based on the solubility and polarity of two drugs. The solution of drugs was prepared in methanol and used for spotting. Methanol gets vaporized soon after application on to the plate under nitrogen stream. After trying different mobile phase system an ideal system was chosen based on the resolution between compounds. The fixed mobile phase system for the separation of two drugs with an appropriate Rf values. The drugs were scanned at 255 nm after the development.

2.3.2 ***Chamber Saturation (Equilibration time)***

Chamber saturation is done so that equilibration is established eventually between the components of developing solvents and their vapour phase and the formation of secondary solvent fronts could be avoided.

Hence in the current study chamber saturation was taken in to consideration to achieve reproducible Rf values and peak area. The mobile phase was placed on one side of twin trough chamber and shaken well. Different saturation times were maintained for different mobile phase. The chamber saturation time for Toluene: methanol: acetic acid (5: 4: 1 % v/v/v) was 30 minutes.

2.3.*4* ***Selection of Detection Wavelength***

By comparing the spectral characters of SITA and SIM, 255 nm the detection wavelength selected for the method with reference to the spectral confirmation graph.

2.3.5 ***Optimized Chromatographic Conditions***

After conforming with the mobile phase and detection wavelength, the optimized conditions for the method was as follows

Stationary Phase	-	Silica Gel 60 F 254 HPTLC Plates
Mobile Phase	-	Toluene: methanol: acetic acid
Mobile Phase ratio	-	(5: 4: 1 % v/v/v)
Detection	-	CAMAG TLC scanner 3, at 255 nm
Temperature	-	Room Temperature
Chamber	-	Twin trough Chamber
Development Mode	-	Ascending Mode

2.4 Procedure

2.4.1 ***Preparation of Standard Stock Solution***

20 mg of SITA was weighed accurately and transferred into a 100 ml volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (200 ng/µl). 25 mg of SIM were weighed accurately and transferred into a 100 ml volumetric flask and dissolved in methanol, after dissolving, the volume was made up to the mark with methanol (1000 µg/ ml). Further dilution was made by pipetting 4 ml of mother solution into same 100 ml standard flask to acquire a concentration of 40 ng/µl solution.

2.4.2 ***Analysis of sample formulation:***

To determine the SITA & SIM content of tablet formulations, twenty (JUVISYNC) tablets (label claim 40 mg & 100 mg) were weighed, to determine the average weight of the tablets, crushed and mixed using a mortar and pestle. A portion of powder equivalent to the weight of 30 mg of SITA was weighed and transferred into a 100 ml volumetric flask, added a minimum quantity of methanol to dissolved the substance by using ultra sonication for 15 minutes, and made up the volume to 100 ml volumetric flask. Then the content was filtered through Whatmann filter paper No. 41. Further dilutions were made by diluting 1 ml into 10 ml and further dilution was made with mobile phase to obtain 300 ng/µl of SITA which contain 120 ng/µl of SIM theoretically. This solution is used for further analysis. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 30 minutes, six test solutions of formulation were injected and recorded the chromatograms.

1.2 METHOD VALIDATION

The method was validated in compliance with ICH guidelines (ICH, 2005). The following parameters were used for validation of the developed method.

1.2.1 *Linearity and Calibration Curve*

Linear relationship between peak area and concentration of the drugs was evaluated over the concentration range expressed in ng band^{-1} by making five replicate measurements in the concentrations containing the concentrations of 100 - 500 ng/µl SITA and 40 - 200 ng/µl SIM respectively. All the solutions were injected and the chromatograms were recorded at 255 nm.

1.2.2 *Precision*

Precision of the developed method was studied by performing repeatability and intermediate precision studies. The sample application and measurement of peak area was determined by performing six replicate measurements of the same band using a sample solution containing 300 ng band^{-1} of SITA and 120 ng band^{-1} of SIM each.

The repeatability of sample application and measurement of peak area were expressed in terms of relative standard deviation (%RSD) and was found to be very low. The low %RSD for intraday and inter-day assay of SIM & SITA performed in the same laboratory, indicating the ruggedness of the method.

1.2.3 *Limits of detection (LOD) and quantification (LOQ)*

The limits of detection and quantification of the developed method were calculated from the standard deviation of the *y*-intercepts and slope of the calibration curves of SITA and SIM using the formulae as given below.

$$\text{Limits of Detection} = 3\alpha/S$$

$$\text{Limits of Quantification} = 10\alpha/S$$

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted thrice and the sample was run in the solvent system. With the help of peak area of methanol, SD was calculated which was further used to find the exact value of LOD and LOQ. Limits of detection (LOD) and quantification (LOQ) were found.

1.2.4 *Recovery studies*

To each 1 ml of pre analyzed formulation solution (300 ng/µl and 120 ng/µl) added 1, 1.25, 1.5 ml of 24 ng/µl raw material stock solution of SITA and 1,1.25,1.5 ml of 96 ng/µl raw material of SIM into a 10 ml volumetric flasks and made up to the mark with mobile phase. The procedure was repeated as per analysis of formulation in triplet of each concentration. The quantity of drug recovered was calculated by using slope and intercept values from the calibration graph. Values of recovery (%), RSD (%), and SE are listed in Table 6; %RSD was always <1% which indicated that the method is accurate.

RESULTS AND DISCUSSION

An effort was made to develop a simple, precise and accurate method for the simultaneous estimation of Sitagliptin Phosphate and Simvastatin bulk and in Pharmaceutical dosage form by HPTLC method.

The initial separation was based upon the solubility of drugs, the different mobile phase were tried to get the better resolution. The different mixtures of the mobile phase tried were Chloroform : Toluene : Methanol : Glacial Acetic Acid, Chloroform : water : Acetic Acid, Chloroform : Toluene : Methanol : Glacial Acetic Acid and Benzene : Toluene : Methanol : Glacial Acetic Acid with different ratios. After various trials Toluene: methanol: acetic acid: (5:4:1 v/v/v) was selected. With the above selected mobile phase the UV spectra of all the drugs were recorded and overlaid. From the overlaid spectra, at 255 nm both the drugs showed marked absorbance.

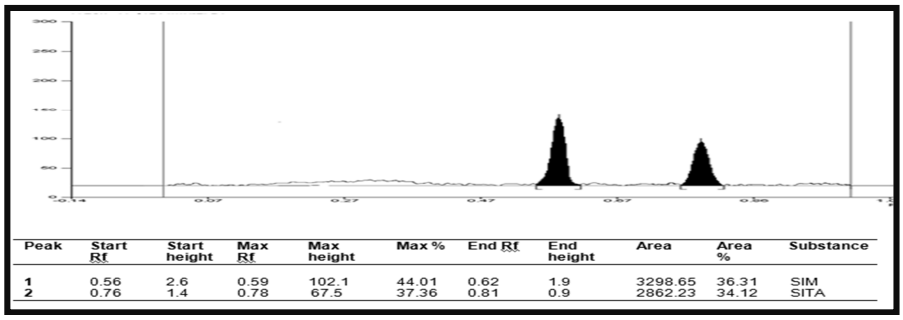
The Rf values for both the drugs were found to be 0.5241 for SITA and 0.7865 for SIM respectively. The linearity range was fixed as 100 – 500 ng/µl for SITA and 40 – 200 ng/µl for SIM in methanol. The calibration graph was recorded using peak area and concentration and these are shown in Figures 4 & 5. The correlation coefficients were found to be 0.99972 for SITA and 0.9997 for SIM respectively.

The optical characteristics such as the Correlation coefficient, Slope, Intercept, LOD and LOQ and were calculated and shown in Table 1. The correlation coefficient values indicated that the selected concentration was linear. The tablet dosage Juvisync was selected for the analysis. The concentration of 300 µg/ml of SITA which is also containing 120 µg/ml of SIM in the mobile phase was prepared. 1 µl spots of each solution were placed on the plates and chromatograms were developed in the twin trough chamber.

The percentage purity of SIM were found to be 99.78 ± 0.632712 and for SITA 99.9830 ± 0.175351. The results of analysis are shown in the Table2 . Precision of the method was confirmed by repeated analysis of formulation for six times. The percentage RSD values were found to be 0.634101 for SIM and 0.175381 for SITA respectively.

The accuracy of the method was confirmed by the recovery studies. The percentage recovery was found to be in the range of 99.82 ± 0.22141 for SIM and 100.11 ± 0.42461for SITA .The percentage RSD values were found to be 0.22182 for SIM and 0.424151 for SITA respectively. The low percentage RSD value indicates that there was no interference due to the excipients used in formulation during the analysis. The data of recovery analysis are listed in Table 2.

Fig: 3 HPTLC CHROMATOGRAM OF SITA & SIM



Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1	0.56	2.6	0.59	102.1	44.01	0.62	1.9	3298.65	36.31	SIM
2	0.76	1.4	0.78	67.5	37.36	0.81	0.9	2862.23	34.12	SITA

Fig: 4 Calibration Curve for SITA

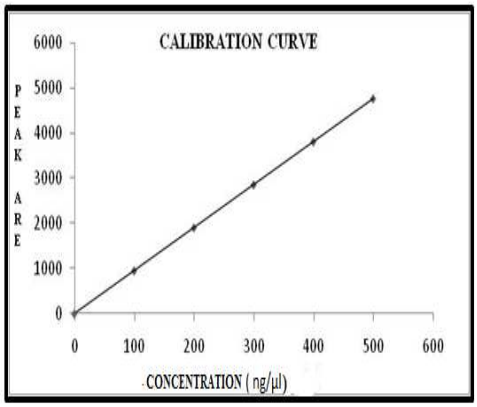


Fig: 5 Calibration Curve for SIM

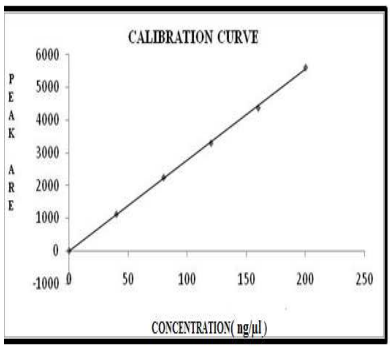


Table: 1 Optical Character of SIM & SITA

Parameters	Sitagliptin phosphate 277 nm	Simvastatin 238 nm
Beer's law limit (µg/ ml)	100-500	40-200
Correlation coefficient (r)	0.9997	0.9997
Régression équation (y = mx+c)	Y = 9.4890x+6.01571	Y = 27.774 - 3.4238
Slope (m)	9.4890	27.7745
Intercept (c)	-6.0157	-3.423
LOD (µg/ ml)	1.71067	0.182
LOQ (µg/ ml)	5.183	0.553
Standard error	46.891	54.491

Summary of regression analysis and validation data

Table: 2: Analysis of formulation

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
SITA	1	40	40.20	100.5	99.78	0.6327	0.6341	0.01757
	2	40	39.94	99.82				
	3	40	39.62	99.07				
SITA	1	100	100.24	100.245	99.983	0.1753	0.1753	0.0048
	2	100	99.72	99.72				
	3	100	99.99	99.99				

Table: 3 Recovery study of the method (using the standard addition method)

Drug	Sample No.	Amount present (µg/ ml)	Amount added	% Recovery*	S.D	% R.S.D	S.E.
SITA	1	200.02	80%	99.57	0.60720	0.6065	0.0674
	2	200.02	100%	100.765			
	3	200.02	120%	99.98			
				100.105			
SIM	1	80.01	80%	100.12	0.75719	0.07565	0.0084
	2	80.01	100%	100.14			
	3	80.01	120%	100.00			
				100.086			

Table: 4 Inter day and Intraday Analysis

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
SIM	1	40	99.65	99.97	0.464901	0.08544	0.464082	0.085474
	2	40	100.53	100.04				
	3	40	100.35	99.87				
Mean			**100.17**	**99.96**				
SITA	1	100	99.85	99.68	0.241937	0.225389	0.241848	0.225524
	2	100	99.95	100.06				
	3	100	100.31	100.08				
Mean			100.0367	99.94				

CONCLUSION

A combination of SIM and SITA is currently available for the treatment of diabetes mellitus. As there are no reported methods for their simultaneous estimation, high performance thin layer chromatography(HPTLC) method was developed and validated for the determination of SITA and SIM in co-formulations on pre-coated silica gel HPTLC plates using chloroform: ethyl acetate: acetic acid (4:6:0.1 v/v/v) as the mobile phase with densitometric detection at 216 nm. The developed method was found to be simple, rapid, selective, sensitive and suitable for simultaneous determination of SIM & SITA. The HPTLC method offers several advantages over liquid chromatographic methods such as the possibility of simultaneous analysis of sample and standard on the same plate, short system equilibrium time, multiple/repeated scanning of chromatograms, higher mobile phase pH, large sample capacity, short run time, minimum solution consumption and no prior treatment for solvents like filtration and degassing. The method was validated in accordance with ICH guidelines (ICH, 2005). The method seems to be suitable for the quality control in the pharmaceutical industry and also for routine in vivo study because of its high sensitivity, simplicity, and selectivity.

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REFERENCES

[1] Szepei Gabor.HPLC in pharmaceutical Analysis, Volume I, 101-173. **(1990).**
[2] Jeffery G.H, Bassett J, Vogel's textbook of Quantitative Chemical Analysis, 5th edition, **(1991).** 217-235.
[3] www.pharmaarticles.net/exclusive/technical/basic-principles-of-hptlc.html.

[4] Sethi P. D. HPTLC – High Performance Thin Layer Chromatography, First Edition, CBS Publisher, New Delhi, 4-66.
[5] Validation of Analytical Procedures: Methodology, ICH Harmonised Tripartite Guidelines, **(2000)**. 1-8.
[6] Willard Hobart. H., Merritt L.L., Dean John. A.,**.** Instrumental Methods of Analysis, 7th edition, CBS Publishers **2008,** 580-610.
[7] Tripathi K.D. Essentials of Medical Pharmacology, 6th edition, Jaypee Publications, New Delhi, 143, 196, 205
[8] Douglas A. Skoog, James Holler F and Timothy A. Nieman. Principles of Instrumental Analysis, 5th edition, Thomson Business Information India Pvt. Ltd., New Delhi, **2006.**331
[9] The British Pharmacopoeia Volume I, International edition, Office of British Pharmacopoeia Commission, London, **2009.** 265.
[10] The Indian Pharmacopoeia Volume I and Volume II, The Indian Pharmacopoeia Commission, 3rd edition, Ghaziabad, **2007.** 359,450.
[11] **(**www.drugbank.com)
[12] The Merck Index, 14th edition, Merck Research Laboratories a Division of Merck & Co. Inc, White house Station, NJ, USA, **2006.** 1465, and 1591.
[13] William Kemp Organic Spectroscopy, 3rd edition, Palgrave Publishers, New York, **2006.** 257-258.
[14] Safaa M Riad, Mamdouh R Rezk, Ghada Y Mahmoud and Abdel-Aziz El Bayoumi Abdel Aleem. *International Journal of Comprehensive Pharmacy*, **2012.** 3(5).
[15] T. Raja, A. Lakshmana Rao., *Rasayan J.Chem*, Vol. 5(3), **2012.** 407- 413.
[16] Jain Pritam, Chaudhari Amar, Desai Bhargav, Patel Shani, Patel Santsaran, Shimpi Hiren., *International Journal of Drug Development & Research*,3(4), **2011**. 194-198.
[17] T. Raja and A. Lakshmana Rao., *IJPCBS*, 2(4), **2012.** 696-702**.**
[18] Hitesh P. Inamdar, Ashok A. Mhaske., *IJPSR*,3(9), **2012.** 3267-3276.
[19] Sheetal Sharma, Nimita Manocha, Priya Bhandari, Sohail Harsoliya, Prabhat Jain.,. *International Journal of Pharmaceutical & Biological Archives*, 3(3), **2012.** 673-678.
[20] Swati Kupkar, Shailaja Jadhav, Vaibhavi Kunjir, Praveen Chaudhari., *Journal of Pharmacy Research,* 5(1), **2012.** 580-582.
[21] Dhiraj Kumar, R.Lavanya, P.Parijata Reddy, Mandeep Kaur, N.Naveen Kumar., *Journal of Pharmacy Research*, 5(8), **2012.** 4421- 4424.
[22] Anilkumar Voodikala, Arunakumari Vanka, Saikumar V Simhadri, Srinivasa Rao Atla, Santoshkumar Tata., *International Journal of Chemical and Analytical Science*, 3(11), **2012.** 1611-1614.
[23] Ankur Kothari, Sheetal Sharma. *Int J Pharm*, 2(3), **2012.** 609-612.
[24] Narendra Nyola, Govinda Samy Jeyabalan.. *World Journal of Pharmacy and Pharmaceutical Sciences*, 1(4), **2012** 1392-1401.
[25] Amruta B. Loni, Minal R. Ghante, S. D. Sawant., *Der Pharma Chemica*, 4 (3), **2012.** 854-859.
[26] N.Monila, Ravi Pratap Pulla, Harshini Shabad, V.Swathi, J.Rajasekhar, A.Ramesh, B.Koti Reddy, B.Umashankar. *IOSR Journal of Pharmacy and Biological Sciences*, 1(6), **2012.** 37-40.
[27] G Jeyabalan, Narendra Nyola., *RRJPA*, 1(1), **2012.** 19-23.
[28] Gebremriam Enema, D Gowris Sankar. *Inventi Impact: Pharm Analysis & Quality Assurance*, **2012.** 4(15).