Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

APPLICATION OF QBD BASED APPROACH IN METHOD DEVELOPMENT OF RP-HPLC FOR SIMULTANEOUS ESTIMATION OF ANTIDIABETIC DRUGS IN PHARMACEUTICAL DOSAGE FORM

[1]PITTU K CHAITANYA REDDY and [2]Dr. PRIYANKA MATHUR

[1]Research Scholar, Bhagwant University, Ajmer, Rajasthan, INDIA.

[2]Faculty of Life Science and Applied Science, Bhagwant University, Ajmer, Rajasthan, INDIA

ABSTRACT

Quality by Design (QbD) refers to the achievement of certain predictable quality with a predetermined and desired specification. The current studies detail QbD enable development of a simple, rapid, sensitive and cost-effective High-Performance Liquid Chromatographic method for the determination of Metformin and Linagliptin in pharmaceutical dosage forms. The factor screening studies were performed using 3-factor 21-run 2-level factorial design. System thematic optimization was performed employing split-plot design by selecting the conc of buffer, pH, and Flow rate as the critical method parameter (CMPs) identified from screening studies, thus evaluating a critical analytical attributes (CAAs) namely, retention time, peak tailing, and resolution The separation was achieved on a Thermo Scientific BDS column (250 × 4.6 mm i.d, particle size of 5 µ) using a mixture of 10 mM ammonium acetate adjusted to pH 3.3 using ortho phosphoric acid) and acetonitrile in the ratio of 35:65 %v/v as mobile phase in an isocratic elution mode, at a flow rate of 1.0 ml/min. The detection was monitored at 255 nm. The retention times of Metformin and Linagliptin were found to be 2.4 min and 4.0 min respectively. Excellent linearity range was found between 150-650 µg/ml for Metformin and 0.5-5.5 µg/ml for Linagliptin..

Keywords: QBD, Metformin, Linagliptin, RP-HPLC method

1. INTRODUCTION

Metformin hydrochloride **(Fig.no.1)** is 1, 1-Dimethyl biguanide monohydrochloride. It is the first-line medication for the treatment of type 2 diabetes. It is a biguanide ant hyperglycemic agent used for treating noninsulin 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 ant hyperglycemic agent that is not associated with weight gain

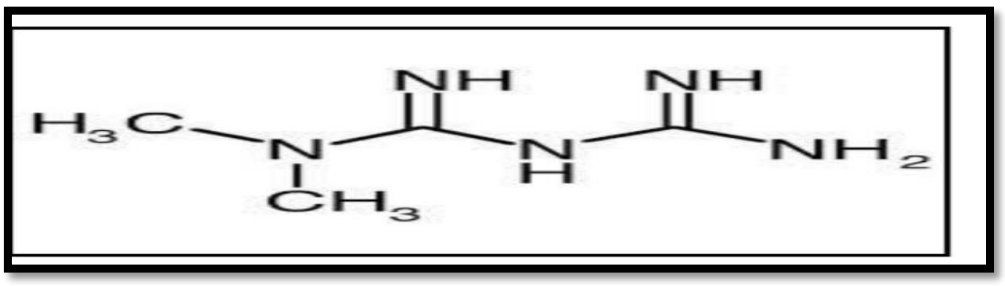


Figure 1 Structure of Metformin

Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

Linagliptin is an oral hypoglycemic drug of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class. Its chemical name is 8-[(3R)-3-aminopiperidin-1-yl]- 7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)- methyl]-4,5-dihydropurine-2,6-dione (Figure no.2). This enzyme inhibiting drug is to be used either alone as an adjunct to diet and exercise or in combination with metformin or a thiazolidinedione to improve glycemic control in adults with type 2 diabetes mellitus. It competitively inhibits an enzyme, dipeptidyl peptidase-4 (DPP-4) increased number of active incretins, i.e., glucagon like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptides (GIP), which in turn reduce the release of glucagon and increase the release of insulin.

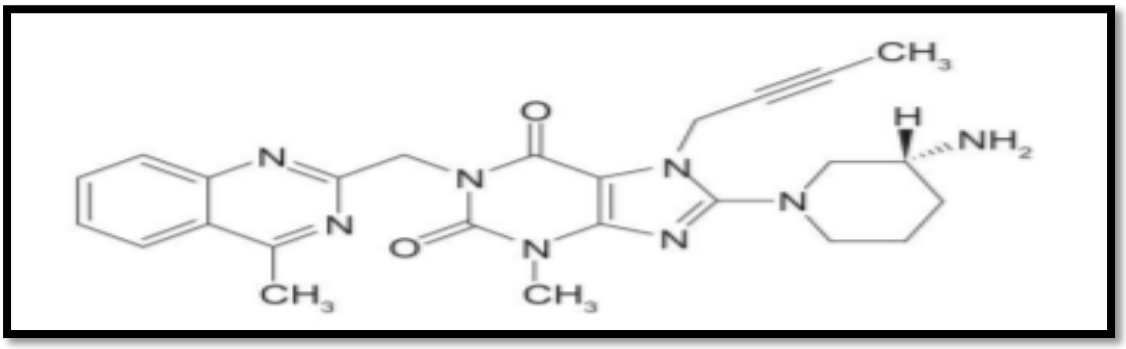


Figure 2 structure of Linagliptin

2. MATERIALS AND METHODS

Apparatus: The HPLC waters 2690/5 liquid chromatography equipped with a PDA detector , the software installed was Empower, with 20µl loop, Hypersil-BDS C18 column (250mmx4.6mm,5µl).The other instrument included are(SARTORIOUS) electronic balance and a sonicator (Fast clean).

Chemicals and reagents

Acetonitrile Methanol Ortho phosphoric acid – HPLC grade Water for HPLC. All the above chemicals and solvents were supplied by S.D. Fine Chemicals Ltd., India; and Qualigens Fine Chemicals Ltd., Mumbai, India, Merck specialties private limited, Mumbai and Ranbaxy Chemicals Ltd., New Delhi, India.

Preparation of standard solutions

Stock solutions were prepared by dissolving 40mg of Metformin 2mg of Linagliptin in 100 ml of mobile phase separately. Aliquots of the standard stock solutions of Metformin and Linagliptin were transferred into 10 ml volumetric flasks and solution was made up to the volume to yield required concentrations of both drugs within the linearity range.

Preparation of sample solutions

Five JENTADUETO D2 tablets each containing 2.5 mg of Linagliptin and 500 mg of Metformin were weighed, average weight was calculated and powdered. A quantity equivalent to 2.5 mg of linagliptin and 500 mg of Metformin was weighed and transferred

Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

into 250 ml volumetric flask. It is extracted with mobile phase. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with mobile phase and filtered. Suitable aliquots of formulation solution were prepared and injected to HPLC to obtain concentration in the linearity range.

METHOD DEVELOPMENT

Waters Photodiode array detector attached to Waters HPLC, which is having Rheodyne injector and auto sampler opted for chromatography. A degasser to remove the dissolved air and column oven to maintain the desired temperature is also available in the system.

The mobile phase system consisting of 0.01mM KH_2Po_4 (pH adjusted to 3.3 with 0.1% ortho phosphoric acid) and Acetonitrile in 40:60 % v/v. Flow rate of the mobile phase was 1.0 ml min-1 and all chromatographic experiments were performed at room temperature (25 ºC ± 2 ºC). Detector wavelength was fixed at 250 nm.

Optimized Chromatogram shown in **Fig no.3**

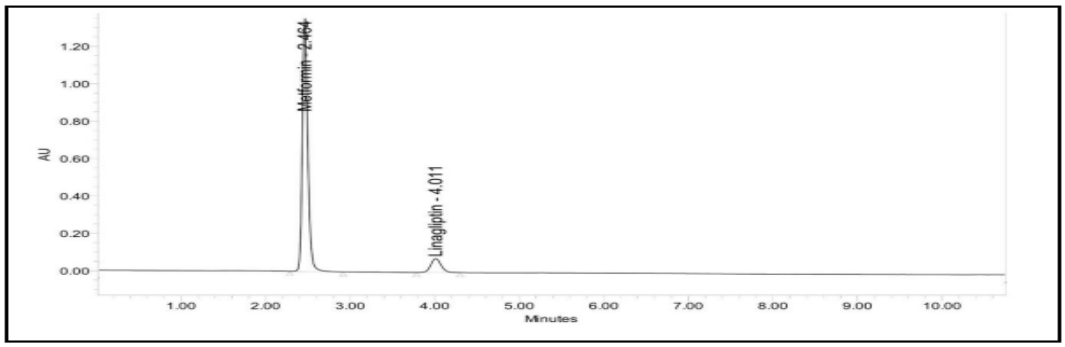


Fig no.3 Optimized Chromatogram

VALIDATION OF ANALYTICAL METHOD

1. Linearity and range

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 150-650 µg/ml for Metformin and 0.5-5.5 µg/ml for Linagliptin, Fig3&4. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves.

2. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated mathematically. The LOD of Metformin and Linagliptin were found to be 0.28µg/ml and 0.08µg/ml respectively. The LOQ of Metformin and Linagliptin were found to be 0.77µg/ml and 0.17µg/ml respectively.

Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

3. PRECISION

A. Intraday precision and inter day precision

Intraday precision was done by carrying out analysis of standard drug solutions at two different concentrations in the linearity range for three times on the same day and %RSD was calculated shown in **Table no. 1**, Inter day precision was done by carrying out the analysis of standard drug solutions at two different concentrations in the linearity range for three days over a period of one week and %RSD was calculated shown in **Table no.2**.

4. Accuracy

Recovery studies of the drug were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out at 50 and 100% levels. Results of recovery are shown in **Table no. 3**

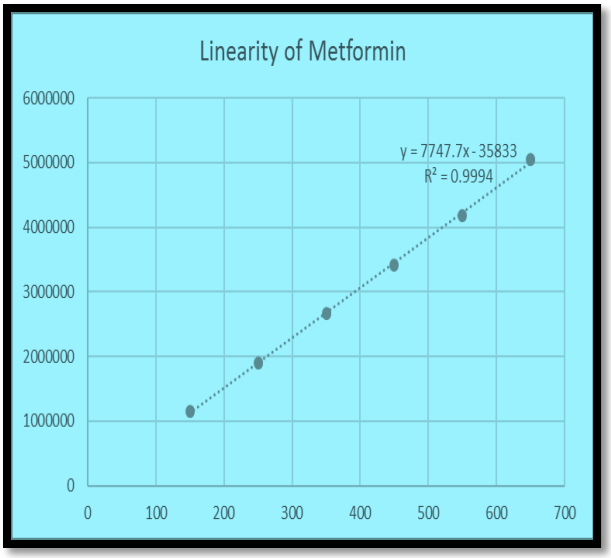


Figure no.3: Linearity of Metformin

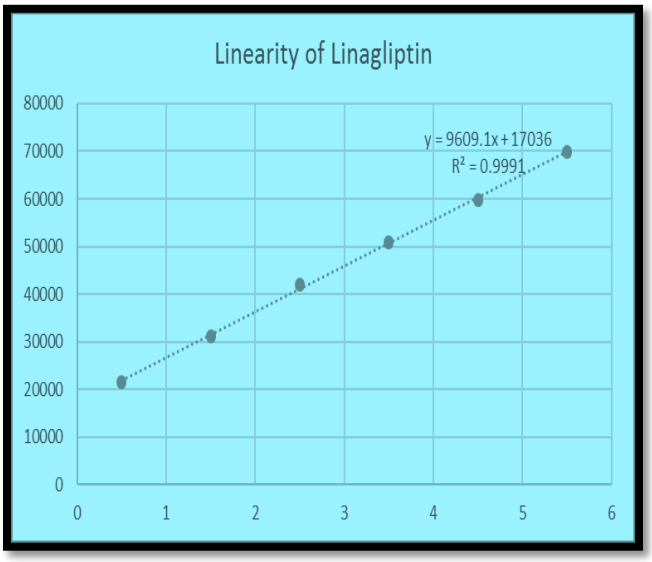


Figure no. 4: Linearity of Lina gliptin

Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

S.NO	Concentration ((µg/ml)		Peak area		%RSD	
	MET	LIN	MET	LIN	MET	LIN
1	250	1.5	1899556	30124	0.59	0.47
			1894556	30144		
			1895756	30255		
2	350	2.5	2675444	42012	0.74	0.51
			2695547	42415		
			2667812	42524		
3	450	3.5	3452544	49856	0.62	0.51
			3421457	49787		
			3427558	49885		

Table-1 Results of intraday

S.NO	Concentration ((µg/ml)		Peak area		%RSD	
	MET	LIN	MET	LIN	MET	LIN
1	250	1.5	1899556	30124	0.49	0.65
			1895875	30547		
			1895778	30257		
2	350	2.5	2675745	42854	0.45	0.85
			2694585	42457		
			2657854	42854		
3	450	3,5	3475844	49856	0.39	0.58
			3425585	49584		
			3428547	49584		

Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

Table: 2 Results of intraday precision

S.NO	Concentration ((µg/ml)		Peak area		%RSD	
	MET	LIN	MET	LIN	MET	LIN
1	250	1.5	1899556 1895875 1895778	30124 30547 30257	0.49	0.65
2	350	2.5	2675745 2694585 2657854	42854 42457 42854	0.45	0.85
3	450	3,5	3475844 3425585 3428547	49856 49584 49584	0.39	0.58

Table 3 Results of accuracy

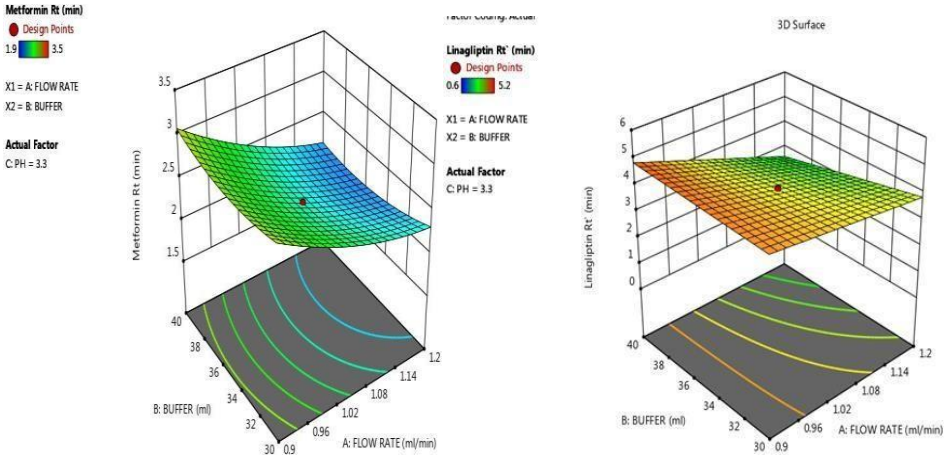
Optimization Data Analysis and Model Validation:

The optimization data analysis was carriedout by multiple linear regression analysis using Design Expert® ver.12 software (M/s Stat-Ease Inc., MN, USA) for fitting the experimental data to the second-order quadratic polynomial model for estimating both the main effects and interaction effects. The model coefficients with statistical significance <0.05 were considered in framing the polynomial equation. The model was finally ratified by analyzing various parameters like coefficient of correlation (r^2). Response surface analysis was carried out from the 3D-response surface plots to discern the factor- response relationship and plausible interaction effect(s) if any. Search for the optimum chromatographic solution was carried out to obtain efficient method performance by numerical optimization and desirability function by “trading-off” of various CAAs as per the desired acceptance criteria, *i.e*., minimization of retention time and peak tailing, and maximization of resolution, respectively. On the heels of numerical optimization, the graphical optimization was also carried out to embark upon the analytical design space and location of the optimized solution.

Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

Std	Run	Factor 1 A:FLOW RATE ml/min	Factor 2 B:BUFFER ml	Factor 3 C:PH	Response 1 Metformin Rt min	Response 2 Linagliptin Rt` min	Response 3 Met Talining fac...	Response 4 Linagliptin Tailin...	Response 5 Resolution
14	1	1.05	35	3.80454	2.5	4.2	1.1	1	5
4	2	1.2	40	3	2.1	3.6	1.3	1.5	4
2	3	1.2	30	3	2.1	3.7	1.3	1.5	4
13	4	1.05	35	2.79546	2.2	3.9	1.2	1.1	5
6	5	1.2	30	3.6	2.9	4	1.1	1.2	5
1	6	0.9	30	3	3.1	4.9	1.5	1.3	4
8	7	1.2	40	3.6	2.1	0.6	1.3	1.1	4
3	8	0.9	40	3	3	4.8	1.2	1.2	3
17	9	1.05	35	3.3	2.4	4	0.9	1.1	9
15	10	1.05	35	3.3	2.4	4	0.9	1.1	9
19	11	1.05	35	3.3	2.4	4	0.9	1.1	9
11	12	1.05	26.591	3.3	2.5	4.1	1	1.2	6
7	13	0.9	40	3.6	2.9	4.2	1.1	1.2	5
16	14	1.05	35	3.3	2.4	4	0.9	1.2	9
12	15	1.05	43.409	3.3	2.9	4.5	1.2	1.5	5
20	16	1.05	35	3.3	2.4	4	0.9	1.1	9
10	17	1.30227	35	3.3	1.9	3.2	1.5	1.3	2
18	18	1.05	35	3.3	2.4	4	0.9	1.1	9
5	19	0.9	30	3.6	3.2	4.8	1.2	1.3	4
9	20	0.797731	35	3.3	3.5	5.2	1.6	1.4	2

Table 4: Results of optimization trails



Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

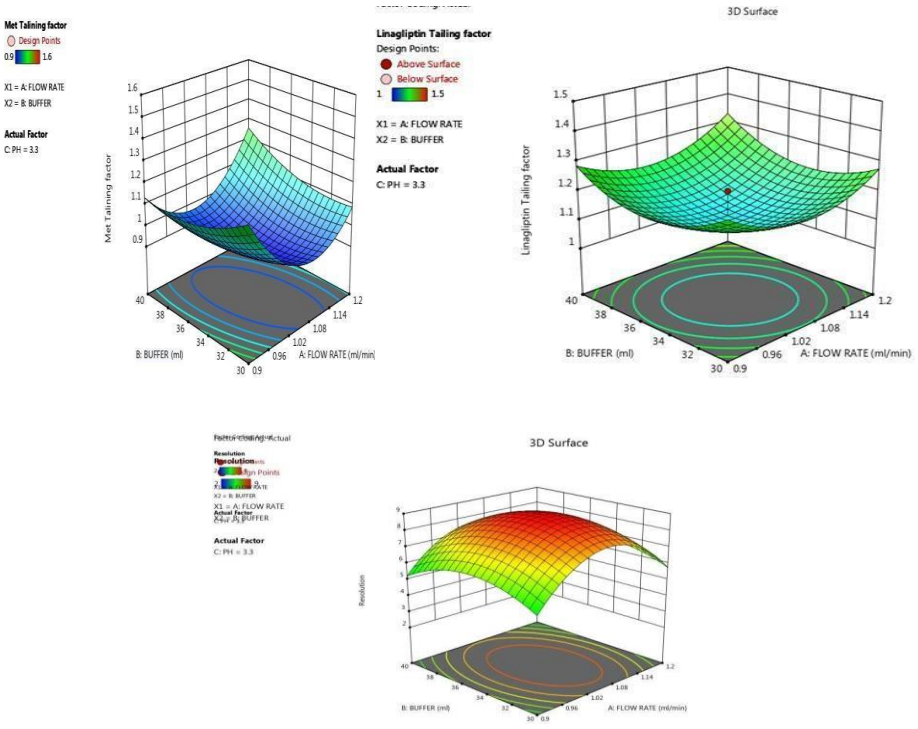


Figure 4: 3d response surface plot depicting the influence of mobile phase ratio, flow rate, buffer as the response variable

CONCLUSION

The developed isocratic Reverse Phase-HPLC method offers simplicity, selectivity, precision and accuracy. In this proposed method symmetrical peaks with good resolution were obtained. The applied BBD design for optimization of Robustness parameters was found to be highly suitable for validation and able to predict minor changes in the flow rate and mobile phase composition for the response.

REFERENCES

1. Asian Guideline for Validation of Analytical Procedure Adopted from ICH guideline, Q2A27 Oct. 1994 and ICH Q2B, 6th Nov A. 1994.
2. Ermer, “Quality by Design in Pharmaceutical Analysis,” Conference organized by European Compliance Academy, Vienna, Austria, 1-2 October 2013,
3. S. F. Warf, “The state of QbD in the bio-pharmaceutical industry conference criticality Assessment, design space implementation and control, San Francisco, Calif, USA, April2013
4. M. Schweitzer, M. Pohl, M. Hanna-Brown et al., “Implications and opportunities of

ISSN:1673-064X
E-Publication:Online Open Access
Vol: 65 Issue 04 | 2022
DOI 10.17605/OSF.IO/WJX6S

Applying QbD principles to analytical measurements," Pharmaceutical Technology, vol. 34, no. 2, pp. 52–59, 2010.

5. P. Elder and P. Borman, "Improving analytical method reliability across the entire product lifecycle using QbD approaches," Pharmaceutical Outsourcing, vol. 14, no. 4, pp. 14–19, 2013.
6. http://www.drugbank.ca/drugs/DB00967.
7. http://www.drugbank.ca/drugs/DB00852
8. Ameeduzzafar, Ibrahim El-Bagory, (2020) Quality by design (QbD) based development and validation of bioanalytical RP-HPLC method for dapagliflozin: Forced degradation and preclinical pharmacokinetic study, Journal of Liquid Chromatography & Related Technologies, 43:1-2, 53-65
9. Vedantika Das, Bhushan Bhairav, R. B. Saudagar. Quality by Design approaches to Analytical Method Development. Research J. Pharm. and Tech. 2017; 10(9): 3188- 3194
10. Thamma Narendra, "Rp-hplc method for simultaneous estimation and stability indicating study of metformin and linagliptin in pure and pharmaceutical dosage forms". International journal of pharmacy and pharmaceutical sciences, vol. 7, no. 3, mar. 2015, pp. 191-7
11. Madhukar. Simple and sensitive analytical method development and validation of metformin hydrochloride by RP-HPLC Int j Pharm sci, vol 3, issue 3, 2011, 117-120
12. K kavitha " Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form" Journal of Chemical and Pharmaceutical Research 5(1):230-235
13. Janardhan, "Analytical Method development and Method validation for the simultaneous estimation of Metformin HCL and Linagliptin in Bulk and tablet Dosage Form by RP-HPLC Method". Int J Pharm 2013; 3(3): 594-600
14. Shayamala T. RP-HPLC Method for the Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Combined Tablet Dosage Forms. Orient J Chem 2012;28
15. Pradeep Kumar "Development of Validated Stability Indicating Assay Method for Simultaneous Estimation of Metformin Hydrochloride and Vildagliptin" by RP-HPLC 2013 Drug Research 64(3)
16. Deepti Jain, Surendra Jain, Deepak Jain, Maulik Amin, Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glimepiride by RP-HPLC in Tablet Formulation, Journal of Chromatographic Science, Volume 46, Issue 6, Pages 501–504
17. Badugu, Lakshman Raju. "A Validated RP-HPLC Method for the Determination of Linagliptin." (2012).
18. Chhetri HP, Thapa P, Van Schepdael A. Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. Saudi Pharm J. 2014 Nov;22(5):483-7. doi: 10.1016/j.jsps.2013
19. Sujatha k "RP-HPLC method for simultaneous estimation of metformin and linagliptin in tablet dosage form" rasayan j. chem vol. 8 | no.4 |426 - 432 /2015