



www.manuscriptscientific.com

MANUSCRIPT
Scientific Services...

Journal of Pharmaceuticals Sciences and Drug Discovery

Original Research Article

Quantitative Computation of Synthetic Mixture Comprising Sitagliptin and Dapagliflozin from Synthetic Mixture by Vierdot's Method

Pinak Patel^{1*}, Yesha Patel¹, Shivani Jani¹ and Krunal Detholia²

¹Department of Pharmaceutical Quality Assurance, Smt. S.M. Shah Pharmacy College, Amsaran, Gujarat-387130, India

²Department of Pharmaceutics, Smt. S.M. Shah Pharmacy College, Amsaran, Gujarat-387130, India.

Corresponding author: Pinak Patel, Department of Pharmaceutical Quality Assurance, Smt. S.M. Shah Pharmacy College, Amsaran, Gujarat-387130, Tel: +91 9913557732; E-mail: pinakqa@gmail.com

Abstract

Current research paper describes highly specific, reproducible and efficient Vierdot's method also known as simultaneous equation method for quantitative computation of synthetic mixture comprising Sitagliptin which is a DPP4 inhibitor and Dapagliflozin which is SGLT2 inhibitor. Both active components belong to the therapeutic class of anti-diabetics. Present UV spectrophotometric method was developed on Shimadzu double beam spectrophotometer equipped with UV probe 2.42 as software using methyl alcohol as solvent. As per the requirements of Vierdot's method, 224 nm and 267 nm was selected as analytical wavelength for quantification of Sitagliptin and Dapagliflozin which is their respective λ_{max} . Method showed linear response in the range of 50-150 $\mu\text{g/mL}$ of Sitagliptin and 5-15 $\mu\text{g/mL}$ of Dapagliflozin. The main requirement of having glen ratio outside the range of 0.1-2.0 was also satisfied at each concentration level. Method was found to be accurate with recovery between 96.8 - 98.2 % for Sitagliptin and 99.6 - 100.8 % for Dapagliflozin. The developed method was validated as per ICHQ2R1 guidelines and was successfully applied for quantitative analysis of synthetic mixture of Sitagliptin and Dapagliflozin.

Keywords: Sitagliptin, Dapagliflozin, Simultaneous equation method, Glen ratio, Analytical method validation

INTRODUCTION

Sitagliptin (SITA) acting as Anti-Diabetic agent (Dipeptidyl peptidase-4 inhibitor) which boosts post prandial insulin release decreases Glucagon secretion and lower mean time as well as fasting blood glucose in type 2 diabetes mellitus. [1]. This agent is used in combination with other oral hypoglycemic agents. Dapagliflozin (DAPA) acts as Sodium-Glucose cotransporter-2(SGLT2) inhibitor. This agent is used in combination with diet and exercise to improve glycemic control in adult with type -2 diabetes mellitus. SGLT 2 is major transporter of glucose whose inhibition induces glycosuria and lower blood sugar in type 2 diabetes mellitus. [2] According to the clinical trial Study of real-World Evidence with SGLT2i (DAPA) and DPP4i (SITA) in Type-2 diabetes mellitus patients in Spain (NCT04149067) which shows beneficial positive effect on patient of Diabetes Mellitus (Type-2) at the close level of 5-10 mg of DAPA and 50-100 mg of SITA. [3] Best clinical effectiveness was observed at 100 mg dose of SITA and 10 mg of DAPA and hence for method development purpose dose was selected as a mixture comprising 10 mg of DAPA and 100 mg of SITA. Several analytical methods are available which can determine SITA and DAPA individually or in combination with another drug [4-23]. From detailed review of literature, it was found that no analytical method is available for determination of DAPA and SITA from simulated mixture or formulation. Furthermore, UV spectrophotometric methods are more convenient with respect to operation in comparison with chromatographic methods of analysis. In additional,

Simultaneous equation method aids virtue of selectivity to the analytical method due to estimation of components at λ_{max} of individuals and the glen ratio was determined. So, for the same reason simultaneous equation method was selected as method of choice from all other multi component UV spectrometric methods.

MATERIALS AND METHOD

Instrument Chemicals and Reagents

DAPA (99.98% pure) and SITA (99.96% pure) were obtained as gift sample for research purpose from, Cadila Healthcare Ltd., Sanand. Methyl alcohol (LR grade) was purchase from S.D. fines. All other excipients required for study were available at research institutions. Spectrophotometric analysis was performed on Shimadzu UV-1800 double beam Spectro photo-meter having path length of 1 cm matched with pair of 10 mm quartz cells. Obtained spectra of SITA and DAPA were overlapped using UV probe 2.42 as software.

Received: April 29, 2022; *Revised:* May 3, 2022; *Accepted:* May 6, 2022

Citation: Patel P, Patel Y, Jani S & Detholia K. (2022) Quantitative Computation of Synthetic Mixture Comprising Sitagliptin and Dapagliflozin from Synthetic Mixture by Vierdot's Method. J Pharm Sci Drug Discov, 1(2):1-5.

Copyright: ©2022 Patel P, Patel Y, Jani S & Detholia K. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Preparation of stock solution and Master stock solution

For the method development purpose, 50 mg of SITA was weighed and diluted to 50 mL (1000 µg/mL) and was further diluted to give final concentration of 250 µg/mL. In similar way 50 mg of DAPA was weighed and diluted to 100 ml (500 µg/mL) and was further diluted to give final concentration of 25 µg/mL.

Selection of analytical wavelength and optimization of model

For selection of Analytical wavelength, working standards of SITA (100 µg/mL) and DAPA (10 µg/mL) was scanned and overlapped with the help UV probe 2.42 software and all criteria for developing simultaneous equation method was assessed at obtained λ_{max} of each drug component. For optimization of simultaneous equation model, absorbance of mixture (10+100 µg/mL) was taken and glen ratio was assessed at proposed concentration level.

Preparation of solution for linearity and range

For method development purpose solution of SITA were prepared in the concentration level of 50 - 150 µg/mL and for DAPA the solution was prepared in the range of 5-15 µg/mL. To adjudge overall linearity, mixture of DAPA and SITA in the ratio of 1:10 (i.e 5+50, 7.5+75, 10+100, 12.5+125 and 15+150 µg/mL) were prepared. To observe the linearity, calibration curve was plotted and regression coefficient was observed.

Intermediate precision (Repeatability)

To adjudge the repeatability of analytical method, solution of linearity studies was analyzed for five time with same conditions. Absorbance was recorded at wavelength of DAPA (224 nm) and SITA (267 nm) for all concentration and relative standard deviation was observed.

Method Precision

Method precision was determined by performing intraday and interday precision. Mixture that represents overall range (5+50, 10+100 and 15+150 µg/mL) were analyzed on same day at different time interval for intraday precision. Mixture that represents overall range (5+50, 10+100 and 15+150 µg/mL) were analyzed on different days for interday precision. To adjudge the method precision, value of RSD was monitored.

Accuracy study

Accuracy of analytical method was adjudged by spiking of placebo with standard solution. Mixture containing 100 mg of directly compressible lactose, 2mg of talc and 2 mg of magnesium stearate was selected as placebo and was spiked at 50, 100 and 150% of target concentration (5+50 µg/mL) (Table 1). Placebo (un spiked) was analyzed at given wavelengths for any possible interference. Each spiked

concentration was analyzed for three times and mean % recovery was observed at each spiked level.

Table 1. Preparation of solutions for accuracy studies (Spiking of placebo with standard).

Unspiked	50%	100%	150%
Placebo	Placebo	Placebo	Placebo
-	Weight accurately 5 mg of DAPA and 50 mg of SITA in 10 ml calibrated volumetric flask	Weight accurately 10 mg of DAPA and 100 mg of SITA in 10 ml calibrated volumetric flask	Weight accurately 15 mg of DAPA and 150 mg of SITA in 10 ml calibrated volumetric flask
Contents diluted up to 10 ml calibrated volumetric flask with Methyl Alcohol and filter the solution			
1 ml of master stock solution further dilute up to 10 ml with Methyl Alcohol			
1 ml filtrate diluted up to 10 ml with Methyl Alcohol	1 ml filtrate diluted up to 10 ml with Methyl Alcohol (5+50 µg/ml)	1 ml filtrate diluted up to 10 ml with Methyl Alcohol (10+100 µg/ml)	1 ml filtrate diluted up to 10 ml with Methyl Alcohol (15+150 µg/ml)

Assay

As the proposed synthetic mixture is having dose of 10 mg of DAPA and 100 mg of SITA, was mixed with selected placebo, and diluted appropriately to give mixture containing 50 µg/ml of SITA and 5 µg/ml of DAPA. This mixture was scanned between 200-400 nm at the wavelength of DAPA and SITA. Absorbance was measured at selected wavelength of DAPA (224 nm) and SITA (267 nm) were transformed to concentration with help of linear regression equation. This mixture was analyzed for three times and mean % assay was drawn.

RESULTS AND DISCUSSION

Selection of analytical wavelength and optimization of model

When the working standards of SITA (x) (50 – 150 µg/mL) and DAPA (y) (5 – 15 µg/mL) were prepared using methyl alcohol as a solvent, 224 (λ_1) nm and 267(λ_2) nm was selected as analytical wavelength as both the components were imposing significant absorbance at that wavelength. 224 and 267 nm was also the λ_{max} DAPA and SITA respectively (Figure 1). Even all criteria for developing simultaneous equation method were satisfied at the mentioned wavelengths (Figure 2). Furthermore, the glen ratio was assessed by taking absorbance of mixture at 224 nm (A_1) and at 267 nm (A_2) at concentration level of 10+ 100 µg/mL of DAPA and SITA, which was found to be lying outside the range of 0.1 to 2. In addition, all prepared mixtures in ratio of 1:10 were analyzed for glen ratio and each glen ratio at each concentration level was found to be outside the range of 0.1 to 2.0.

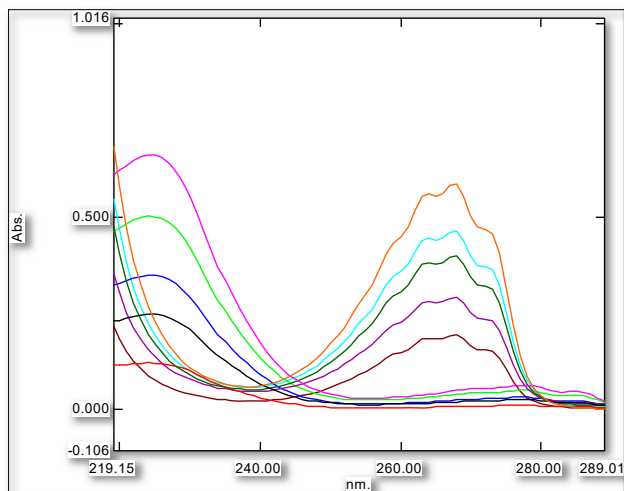


Figure 1. Overlay spectra of individual solution of DAPA and SITA.

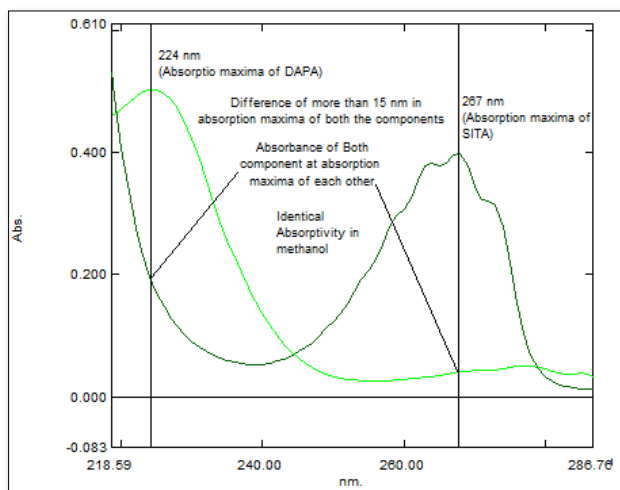


Figure 2. Overlay spectra of DAPA and SITA (10+100 µg/mL).

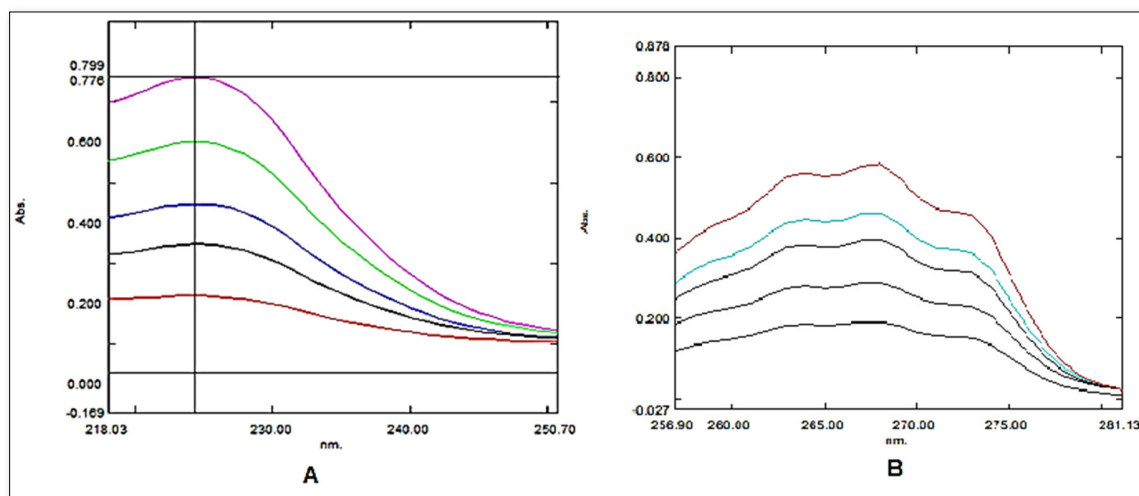


Figure 3. Overlay spectra of Mixture of DAPA and SITA at 224 nm (A) and 267 nm (B).

Analytical Method Validation

All validation parameters were studied as per ICH guidelines.

Linearity and Range

It was found that during observing all concentration of SITA, non-linearity was observed below 25 µg/mL and above 150 µg/mL of SITA and hence range was kept between 50-150 µg/mL. For DAPA the non-linearity was observed above 20 µg/mL and hence by taking ratio of synthetic mixture into consideration (1:10), range of DAPA was kept between 5-15 µg/mL. Beer-Lambert law is also being satisfied at the selected concentration level. When calibration curve was plotted for given concentration range at selected wavelengths, value of linear regression coefficient was found to be 0.991 and 0.991 for SITA at 224 nm and 267 nm respectively. For DAPA, the value of regression coefficient was found to be 0.996 and 0.995 at 224 nm and 267 nm respectively. Regression equation was found to be $y = 0.001 X + 0.020$ for SITA at 224 nm and $y = 0.003 X + 0.0001$ at 267 nm. In the same way regression equation for DAPA was found to be $y = 0.056 X - 0.060$ at 224 nm and $y = 0.003 X + 0.001$ at 267 nm (**Table 2**) value of absorptivity (ax_1 , ax_2 , ay_1 and ay_2) were determined from linear regression equation. All prepared mixtures were also found to be linear with respect to absorbance at 224 nm 267 nm (**Figure 3**). When value of glen ratio was assessed at each concentration level of mixture, the ratio was found to be beyond range of 0.1-2.0, by the same we can conclude that estimation of SITA and DAPA can be done accurately without any interference (**Table 3**).

Table 2. Linearity data of DAPA and SITA at 224 and 267 nm.

Linearity data of SITA				
Concentration (µg/mL)	*At 224 nm		*At 267 nm	
	*Mean Abs. ± SD	RSD	Mean Abs. ± SD	RSD
50	0.095 ± 0.0018	1.969	0.193 ± 0.0037	1.953
75	0.148 ± 0.0025	1.739	0.291 ± 0.0048	1.683
100	0.191 ± 0.0020	1.068	0.400 ± 0.0057	1.437
125	0.222 ± 0.0019	0.865	0.451 ± 0.0062	1.376
150	0.262 ± 0.0019	0.733	0.579 ± 0.0047	0.815
Regression equation	y = 0.001 X + 0.020		y = 0.003 X + 0.0001	
Linearity Data of DAPA				
Concentration (µg/mL)	At 224 nm		At 267 nm	
	*Mean Abs. ± SD	RSD	*Mean Abs. ± SD	RSD
5	0.227 ± 0.002	1.033	0.016 ± 0.0002	1.400
7.5	0.347 ± 0.003	1.018	0.023 ± 0.0002	1.174
10	0.504 ± 0.004	0.859	0.030 ± 0.0003	1.078
12.5	0.664 ± 0.004	0.615	0.039 ± 0.0003	0.914
15	0.777 ± 0.003	0.501	0.045 ± 0.0003	0.814
Regression equation	y = 0.056 X - 0.060		y = 0.003 X + 0.001	

*Mean abs: It is the average of total 5 determination taken at given wavelength.

SD and RSD correspond to standard deviation and relative standard deviation respectively.

Table 3. Linearity data for all standard mixture at 224 and 267 nm with calculated glen ratio.

Concentration of Mixture (µg/mL)	*Mena Abs at 224 nm ± SD	*Mena Abs at 267 nm ± SD	Glen ratio observed
5+50 µg/mL	0.308 ± 0.003	0.197 ± 0.003	3.63
7.5+75 µg/mL	0.497 ± 0.004	0.335 ± 0.003	3.55
10+100 µg/mL	0.665 ± 0.004	0.396 ± 0.003	3.94
12.5+125 µg/mL	0.834 ± 0.005	0.492 ± 0.003	3.95
15+150 µg/mL	0.905 ± 0.004	0.594 ± 0.003	3.58

*Mean abs: It is the average of total 5 determination taken at given wavelength.

SD corresponds to standard deviation.

Repeatability

When all mixtures were analyzed at all concentration, calculated relative standard deviation at each level was found to be less than 2 so that method was found to be repeatable over the range of 50 - 150 µg/ml for SITA and 5 - 15 µg/ml for DAPA. Total five determinations were made at each concentration level.

Method precision

For determining inter day and intraday precision, %RSD was monitored at selected concentration level which was found to be less than 2 so method was found to be precise for estimation of SITA and DAPA.

Accuracy study

The developed method was found to be accurate as value of % recovery at each spiked level was found to be in the range of 98-102. It suggests that the excipients used as placebo were not interfering in the estimation of DAPA and SITA (Table 4), and hence, the developed method was found to be accurate for estimation of DAPA and SITA from synthetic mixture.

Table 4. Accuracy data of SITA and DAPA by simultaneous equation method.

Level of spiking	Total placebo (mg)	Amount of std. drug added (µg/ml)		Amount of drug recovered (µg/ml)		% recovery	
		SITA	DAPA	SITA	DAPA	SITA	DAPA
Unspiked	104	-	-	-	-	-	-
50 %	104	50	5	50.41	4.88	100.8	97.6
100 %	104	100	10	100.84	9.68	100.8	96.8
150 %	104	150	15	149.41	14.73	99.6	98.2

Assay

When prepared synthetic mixture was analyzed by developed and validated method, % assay was found to be 100.0 ± 0.6 for SITA and for 100.1 ± 0.7 DAPA.

SUMMARY AND CONCLUSION

The Simultaneous equation method was developed and validated as per ICHQ2R1 guidelines and was successfully applied for determination of SITA and DAPA from its synthetic mixture. Present method was found to be economical in terms of cost and time. Commonly used excipient didn't interfere in estimation of SITA and DAPA and hence, the developed method was found to be specific, repeatable and precise.

ACKNOWLEDGEMENTS

Authors are extremely grateful to Cadila Healthcare Ltd. For providing gift sample of DAPA and SITA without them this work would not have taken place. We are also thankful to overall research teams who have contributed in either of the way. The authors are also grateful to Smt. S.M. Shah Pharmacy College for providing excellent research facilities and promoting research activities.

REFERENCES

- Ravinandan AP, Basavanagowda GM, Jimmy OD (2014) Review on Sitagliptin. Int J Pharm Res Sch 3(2): 577-581.
- Filippatos TD, Liberopoulos EN, Elisaf MS (2015) Dapagliflozin in patients with type 2 diabetes mellitus.

- Ther Adv Endocrinol Metab 6(1): 29-41.
3. Serge AJ, Hordy E, Sugg J, Parikh S (2014) Dapagliflozin is Effective as add on therapy to Sitagliptin with Metformin: A 24-week, Multicenter, Randomized, Double-blind, Placebo controlled study. *Diabetes Care* 37: 740-750.
 4. Jani BR, Shah KV, Kapupara PP (2015) Development and validation of UV spectroscopic first derivative method for simultaneous estimation of Dapagliflozin and Metformin hydrochloride in synthetic mixture. *J Bioequiv Stud* 1(1): 102.
 5. Bodade BJ, Kande DA, Chaudhari SS (2019) Quantitative Estimation of Dapagliflozin in blood plasma by using UV Spectroscopy. *Pharmaceutica Analytica Acta* 10(2): 2153-2435.
 6. Sanagapati M, Dhanalakshmi K, Kavitha B (2014) Method Development and validation of Dapagliflozin API by UV spectroscopy. *Int J Pharm Sci Rev Res* 27(1): 270-272.
 7. Mante GV, Gupta RK, Hemke AT (2017) Estimation of Dapagliflozin from its Tablet Formulation by UV spectroscopy. *Pharma Methods* 8(2): 102-107.
 8. Sundar PS, Vasanthi R, Algar RM, Urooj A, Rajeswar DK, et al. (2017) Development and validation of RP-HPLC method for simultaneous estimation of Dapagliflozin and Metformin in bulk and in synthetic mixture. *World J Pharm Pharm Sci* 6(7): 2139-1250.
 9. Mohammad Y, Shankar GD (2008) A Validation Stability indicating High Performance Liquid Chromatographic method for simultaneous Determination of Metformin HCl and Dapagliflozin in bulk drug and Tablet dosage form. *Asian J Pharm Clin Res* 8(3): 320-326.
 10. Suma BV, Deveswar R, Premnath Shenoy A (2019) A new High Performance Thin Layer Chromatographic Method Development and Validation of Dapagliflozin in bulk and tablet dosage form. *J Pharm Pharm Sci* 11(8): 58-63.
 11. Mante GV, Hemke AT, Umekae MJ (2018) RP-HPLC Method for estimation of Dapagliflozin from its Tablet. *Int J Chem Tech Res* 11(1): 242-248.
 12. Ashwini R, Mukkanti EM, Srinivasa P (2018) A Novel RP-HPLC method for simultaneous estimation of Dapagliflozin and Saxagliptin in Bulk and Pharmaceutical Dosage form. *Int J Pharm Sci* 9(12): 5167-5167.
 13. Raja T, Rao AL (2012) Validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate in bulk drug and pharmaceutical formulation, *Int J Pharm Chem Biol Sci* 2(4): 696-702.
 14. Mohd Z, Bundel SS, Bharkad VB, Khan HN, Thoke ST (2019) Development and validation of UV spectroscopic method for simultaneous estimation of Dapagliflozin and Saxagliptin in synthetic mixture. *Int J Pharm Anal Res* 8(1): 59-66.
 15. Nashwahgadallah M (2014) Validated HPLC method for simultaneous determination of sitagliptin, metformin and atorvastatin in pure form and in pharmaceutical formulations. *Int J Pharm Pharm Sci* 6(5): 665-670.
 16. Grace AC, Thangavel P, Murugesan J, Srinivasan K, Thangavel S (2017) Analytical method for determination of Sitagliptin: An Updated Review. *Int J Pharm Sci Rev Res* 43(1): 217-225.
 17. Reichal RC, Rao GM (2015) Development and Validation of spectroscopic method for simultaneous estimation of Gliclazide and Sitagliptin Phosphate monohydrate in bulk and pharmaceutical dosage form. *Int J Pharm Pharm Sci* 7(6): 372-376.
 18. Reddy BVR, Raman N, Kumar BS, Chintal R (2013) Chiral separation of Sitagliptin phosphate enantiomer by HPLC using amylose based chiral stationary phase. *J Pharm Res* 7(6): 546-550.
 19. Adsul S, Bidkar J, Harer S (2018) RP- HPLC Method Development and Validation for Simultaneous Estimation for Metformin and Sitagliptin in Bulk and Tablet Formulation. *Int J Chem Tech Res* 11(11): 428-435.
 20. Jalkote RN, Kalshetti MS (2019) UV Spectrophotometric method development and validation for determination of Sitagliptin in API and in pharmaceutical dosage form. *World J Pharm Pharm Sci* 8(5): 916-925.
 21. Raja T, Rao LA (2012) Validation HPTLC method for simultaneous estimation of Metformin hydrochloride and Sitagliptin phosphate in bulk drug and formulation. *Rasayan J Chem* 5(3): 407-413.
 22. Karimulla SK, Vasanth PM, Ramesh T, Ramesh M (2013) Method development and validation of Sitagliptin and Metformin using reverse phase HPLC method in bulk and tablet dosage form. *Der Pharmacia Letter* 5(5): 168-174.
 23. Ganesan A, Tangarasu V (2016) Validated HPTLC method for simultaneous estimation of Sitagliptin phosphate and Simvastatin in tablet dosage form. *Der Pharmacia Letter* 8(4): 6-12.