Journal for ReAttach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 December; Vol 6 (1): 914-921

Method Validation Of Combinational Drug Substances By Ultra Performance Liquid Chromatography

Chowdeswari Darugu[1*], Dr. B. Ananda Kumar[2], Dr.Ch.S. Anuradha[3], Dr.S.Shobha Rani[4*]

[1*]Research Scholar, Department of Chemistry, Andhra University TDR-HUB & Assistant Professor in Chemistry, Sir C R Reddy College of Engineering, Eluru
[2]Lecturer, Department of Chemistry, Sri YN College (A), Narsapur - 534275, West Godavari District, Andhra Pradesh – India
[3]Lecturer in Chemistry, Dr.V.S.Krishna Government Degree and PG College (A), Visakhapatnam -530013 India
[4]Principal, Vishaka Government Degree College for women, Visakhapatnam.

***Corresponding Author:** Chowdeswari Darugu
*Research Scholar, Department of Chemistry, Andhra University TDR-HUB & Assistant Professor in Chemistry, Sir C R Reddy College of Engineering, Eluru

Received date: 01.05.2023 **Revised date:** 07.07.2023 **Accepted date:** 12.08.2023

Abstract:

Combination drug or a fixed-dose combination (FDC) is a medicine that includes two or more active ingredients combined in a single Dosage form. Dapagliflozin, Metformin and Vildagliptin is a combination of three antidiabetic medicines. Dapagliflozin increases urinary glucose excretion and reduces blood glucose levels. Vildagliptin helps control blood sugar levels by increasing substances (incretins) in the body that make the pancreas release more insulin. An effective stability indicating UPLC method has been developed and validated for the determination of Dapagliflozin, Metformin and Vildagliptin in pure and applied successfully.

Key words: Dapagliflozin, Metformin, Vildagliptin, UPLC, method validation

Introduction:

A combination drug or a fixed-dose combination (FDC) is a medicine that includes two or more active ingredients combined in a single dosage form [1]. Terms like "combination drug" or "combination drug product" can be common shorthand for an FDC product (since most combination drug products are currently FDCs), although the latter is more precise if in fact referring to a mass-produced product having a predetermined combination of drugs and respective dosages (as opposed to customized polypharmacy via compounding [2]). And it should also be distinguished from the term "combination product" in medical contexts, which without further specification can refer to products that combine different types of medical products—such as device/drug combinations as opposed to drug/drug combinations [3].
Metformin is prescribed ubiquitously by endocrinologists to tackle type II diabetes mellitus unless contraindicated. It inhibits the mitochondrial respiratory chain in the liver, leading to activation of AMP-activated protein kinase (AMPK), enhancement of insulin sensitivity (via effects on fat metabolism), and lowered cyclic adenosine monophosphate (cAMP) levels, thereby reducing the expression of gluconeogenic enzymes and maintaining euglycemic state [4]. By inhibiting sodium-glucose co-transporter 2 (SGLT2), dapagliflozin blocks the reabsorption of filtered glucose in the kidney, increasing urinary glucose excretion and reducing blood glucose levels. Its mechanism of action is independent of pancreatic β cell function and modulation of insulin sensitivity [5]. Vildagliptin binds covalently to the catalytic site of dipeptidyl peptidase-4 (DPP-4), eliciting prolonged enzyme inhibition. This raises intact glucagon-like peptide-1 (GLP-1) levels, both after meal ingestion and in the fasting state. It has been shown to stimulate insulin secretion and inhibit glucagon secretion in a glucose-dependent manner [6]. Recent studies [7-8] evaluated the efficacy of these drugs as an adjuvant to metformin monotherapy in managing T2DM and provided inconsonant results. Lately, a network meta-analysis [9] showcased the improved efficacy of add-on vildagliptin and dapagliflozin to metformin monotherapy as compared to antidiabetic drugs in the long-term management of diabetes.

Experimental:

UPLC Simultaneous Method Development for Metformin, Dapagliflozin and Vildagliptin

a) Equipment:

Table No.1: List of Apparatus used in UPLC

S.No	Name	Model	Manufacturer
1.	UPLC	ACQUITY	Waters - Empower software2.0versions
2.	pH meter	-	Eutech
3.	Weighing balance	-	Sartouris
4.	UV/VIS spectrophotometer	-	UV-1700
5.	Pipettes, beakers and Burettes	-	Borosil
6.	Ultra sonicator	UCA 701	Unichrome
7.	Pump	Isocratic model	--

(b) Reagents & Chemicals

Table No.2: List of chemicals used in UPLC Method

S.No	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Rankem
2.	Perchloric acid	HPLC	Analytical reagents
3.	Water	HPLC	Milli Q or equivalent

Determination of Working Wavelength (λ_{max}):

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

The wavelength of maximum absorption of the solution of the drugs in mixture of Acetonitrile and Perchloric acid (40:60) were scanned using PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and Perchloric (40:60) as blank. The absorption curve shows isobestic point at 235nm. Thus 235 nm was selected as detector wavelength for the UPLC chromatographic method.

Determination of Working Wavelength (λ_{max}):

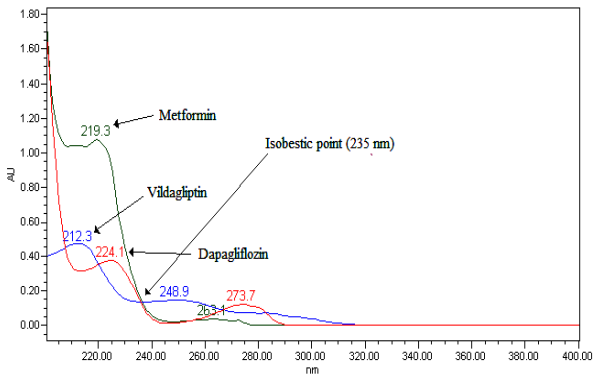

Fig No.:1 PDA - Spectrum of Metformin ,Dapagliflozin and Vildagliptin

Chromatographic conditions:

During the selection of chromatographic conditions, numbers of trails were carried out and the best trail was selected for optimized method.

Preparation of standard stock solution

Accurately weigh and transfer 25 mg of Metformin, 5mg of Dapagliflozin and 5mg Vildagliptin working standard into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1 ml of the Dapagliflozin solutions into a 10 ml volumetric flask and dilute up to the mark with diluent (Stock solution)

Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (250ppm of Metformin, 5ppm of Dapagliflozin,50ppm of Vildagliptin)

Sample Solution Preparation:
Accurately weighed and transfer 35.6mg of Metformin' Dapagliflozinand Vildagliptin sample into a 10mL clean dry volumetric flask add Diluent and sonicate it up to 30 mins to dissolve, and centrifuge for 30min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter. (Stock solution). Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (250ppm of Metformin, 5ppm of Dapagliflozin,50ppm of Vildagliptin).

Chromatographic condition:
Use suitable Ultra Performance Liquid Chromatographic equipped with PDA detector.
Column: C18 (100x 2.1mm, 1.7µm)
Mobile phase ratio: Acetonitrile: 0.1% Perchloric acid (40:60)
Detection wavelength: 235 nm
Flow rate: 0.5ml/min
Injection volume: 5µl
Run time: 3min

LINEARITY:

Preparation of stock solution:
Accurately weigh and transfer 25 mg of Metformin, 5mg of Dapagliflozin and 5mg Vildagliptin working standard into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1 ml of the Dapagliflozin solutions into a 10 ml volumetric flask and dilute up to the mark with diluent (Stock solution)

Preparation of Level – I (62.50ppm of Metformin, 1.25ppm of Dapagliflozin, 12.50ppm of vildagliptin):
0.25 ml of above stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (125ppm of Metformin, 2.50ppm of Dapagliflozin, 25ppm of vildagliptin):
0.5 ml of above stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (187.50ppm of Metformin, 3.75ppm of Dapagliflozin, 37.50ppm of vildagliptin):
0.75 ml of above stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV (250ppm of Metformin, 5ppm of Dapagliflozin, 50ppm of vildagliptin):
1 ml of above stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (312.50ppm of Metformin, 6.25ppm of Dapagliflozin, 62.50ppm of vildagliptin)
1.25 ml of above stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – VI (375ppm of Metformin, 7.50ppm of Dapagliflozin, 75ppm of vildagliptin)
1.5 ml of above stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Results and Discussion:

Trails in optimization of chromatographic condition

Table No.3: TRIAL-1 Chromatographic conditions

Column	Shield RP 18 (50 x 1.0mm, 1.7µm)
Mobile phase ratio	Acetonitrile: 0.1% TFA (20:80)
Detection wavelength	200-400nm
Flow rate	0.5ml/min
Injection volume	10µl
Run time	17min
Observation:	Only two peaks are eluted

Table No.4: TRIAL-2 Chromatographic conditions

Column	Shield RP 18 (50 x 1.0mm, 1.7µm)
Mobile phase ratio	Acetonitrile: 0.1% TFA (30:70)
Detection wavelength	235nm
Flow rate	0.5ml/min
Injection volume	5µl
Run time	17min
Observation	Base line is not sufficient

Optimization of chromatographic conditions

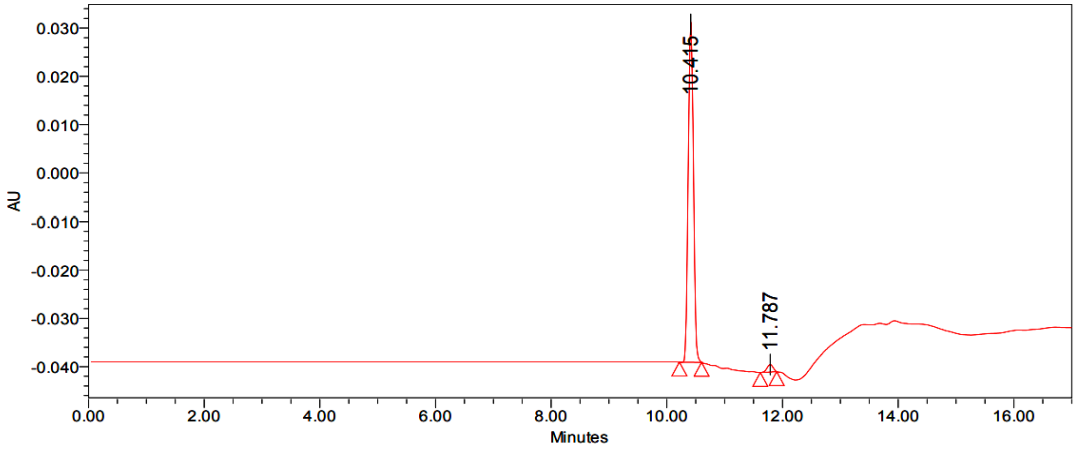

Fig No-2: chromatogram of Trial-1

	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1		10.415	441408		1.03	5481
2		11.787	11215	7.29	0.90	6040

Trial-2:

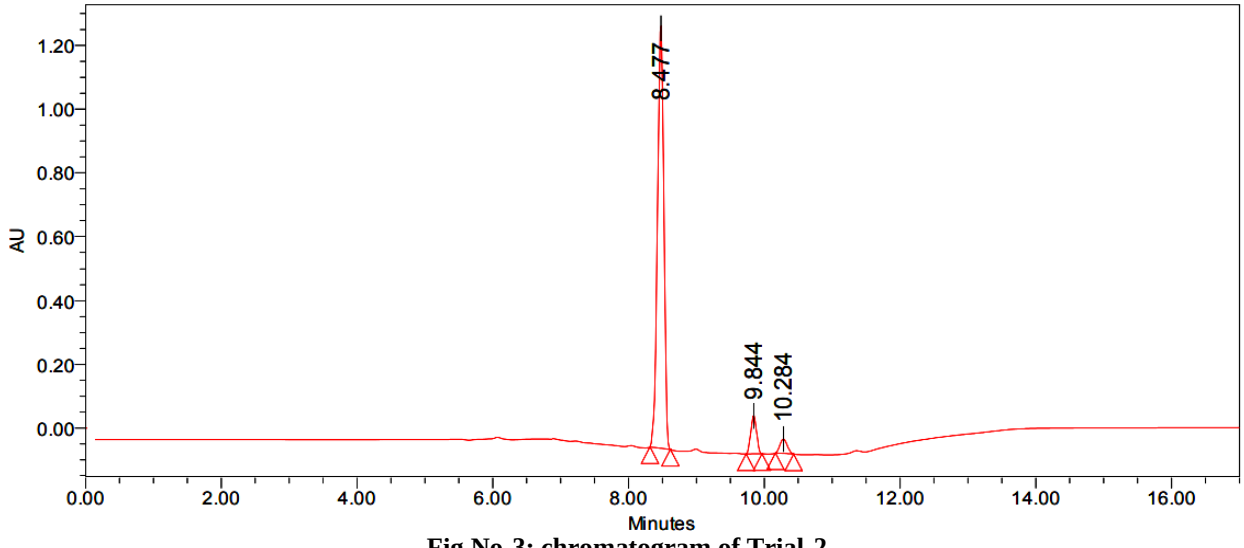

Fig No-3: chromatogram of Trial-2

	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1		8.477	8639184		0.90	13271
2		9.844	714531	7.66	1.01	5889
3		10.284	337676	2.32	1.09	9202

Linearity:

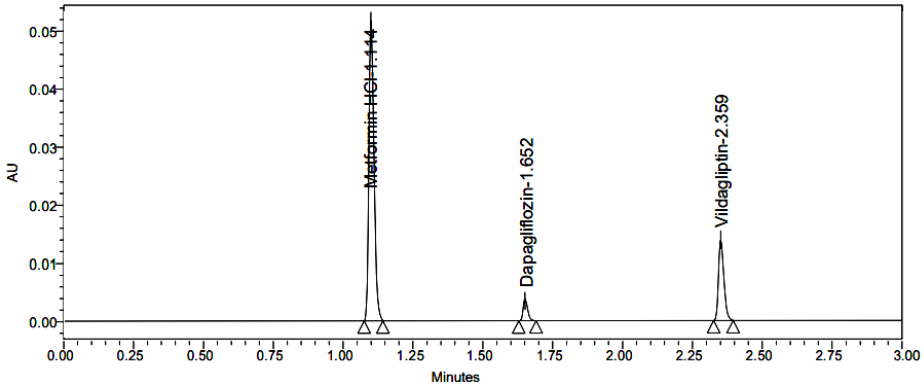

Fig No.4: Chromatogram of Linearity-25%

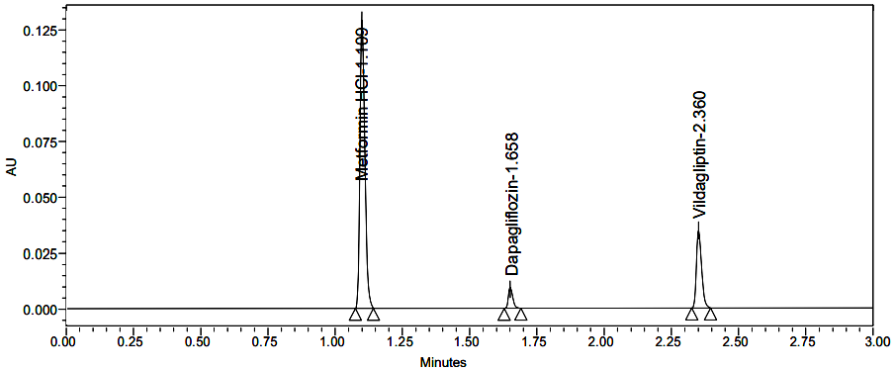

Fig No.5: Chromatogram of Linearity-50%

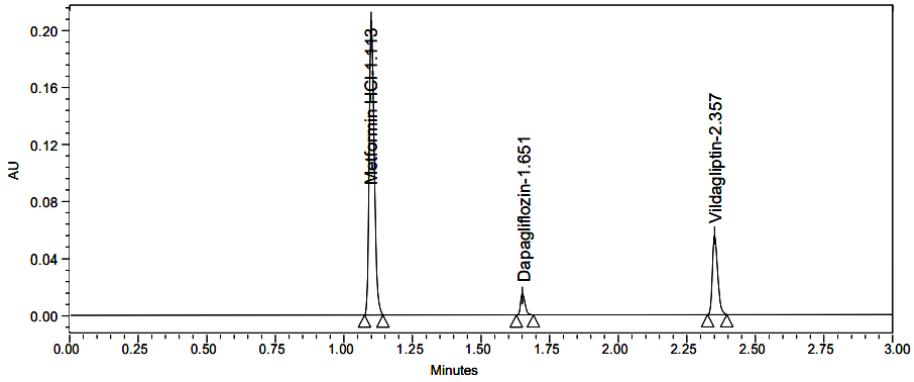

Fig No.6: Chromatogram of Linearity-75%

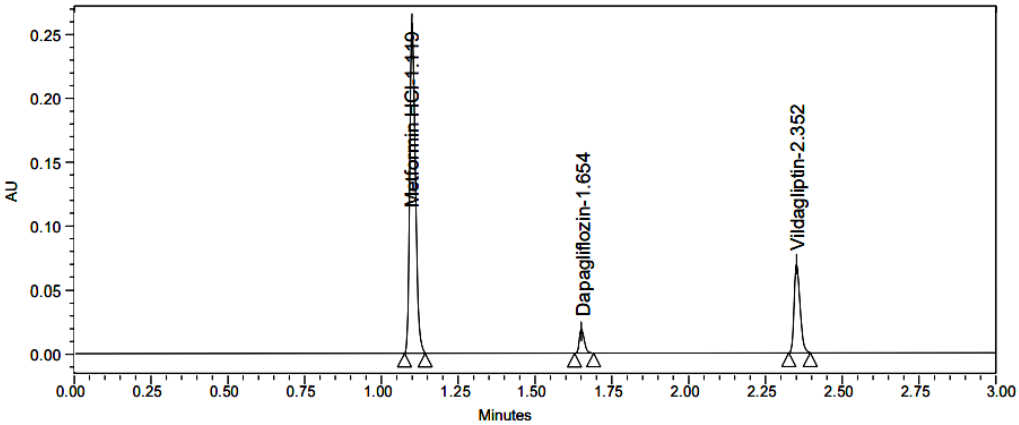

Fig No.7: Chromatogram of Linearity-100%

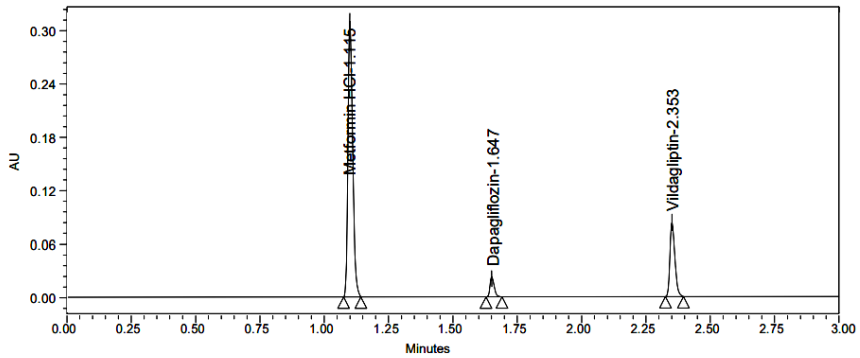

Fig No8: Chromatogram of Linearity-125%

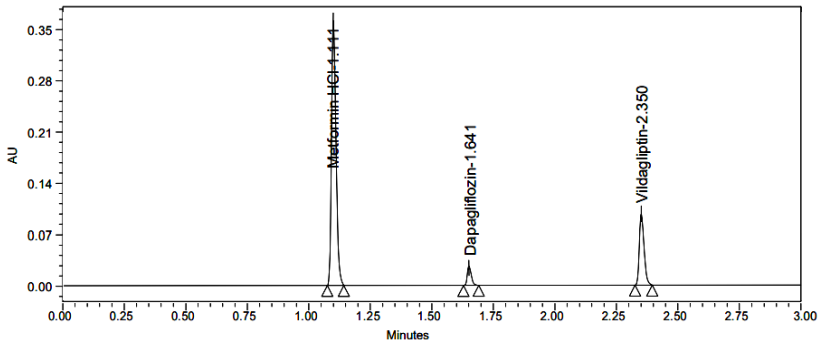

Fig No.9: Chromatogram of Linearity-150%

Table No.5: Results of linearity for Metformin ,Dapagliflozin and Vildagliptin

S.NO	Metformin		Dapagliflozin		Vildagliptin	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	62.50	643702	1.25	12635	12.50	126231
2	125.00	1274980	2.50	25471	25.00	255127
3	187.50	1867131	3.75	39526	37.50	397022
4	250.00	2568724	5.00	51589	50.00	516930
5	312.50	3147612	6.25	64123	62.50	633407
6	375.00	3796028	7.50	76944	75.00	764827
Regression equation	Y=10108.37+4420.14		Y=10283.60+49.07		Y=10201.82+2223.86	
Slope	10108.37		10283.60		10201.82	
Intercept	4420.14		49.07		2223.86	
R^2	0.9987		0.99988		0.99974	

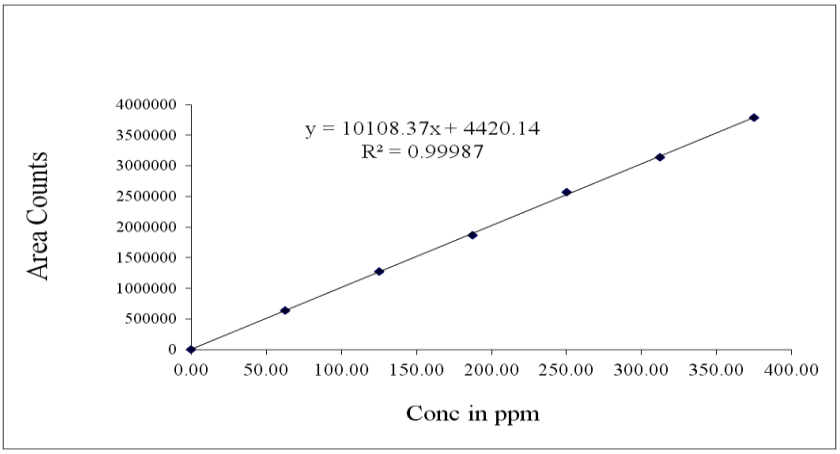

Fig No.10: Calibration curve for Metformin at 235 nm

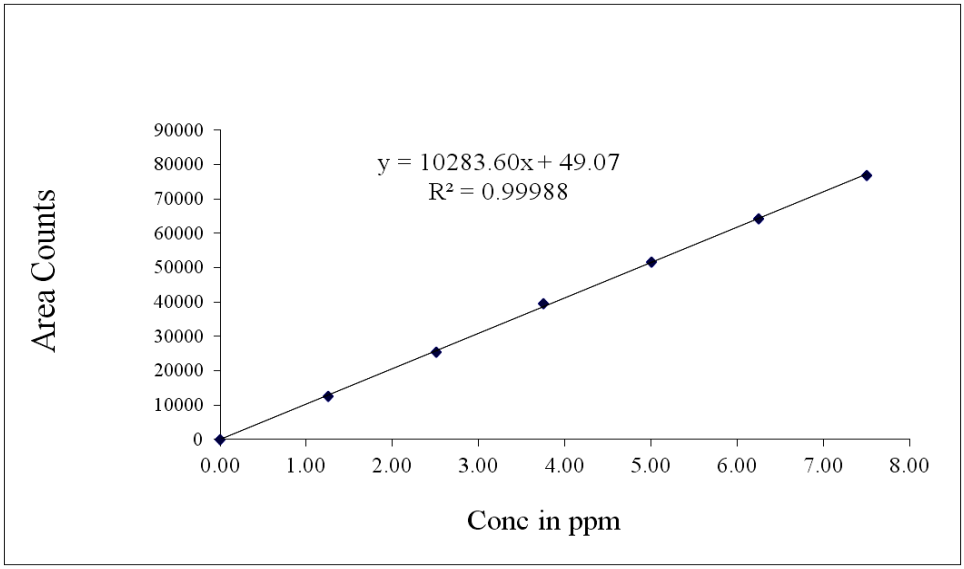

Fig No.11: calibration curve for Dapagliflozin at 235 nm

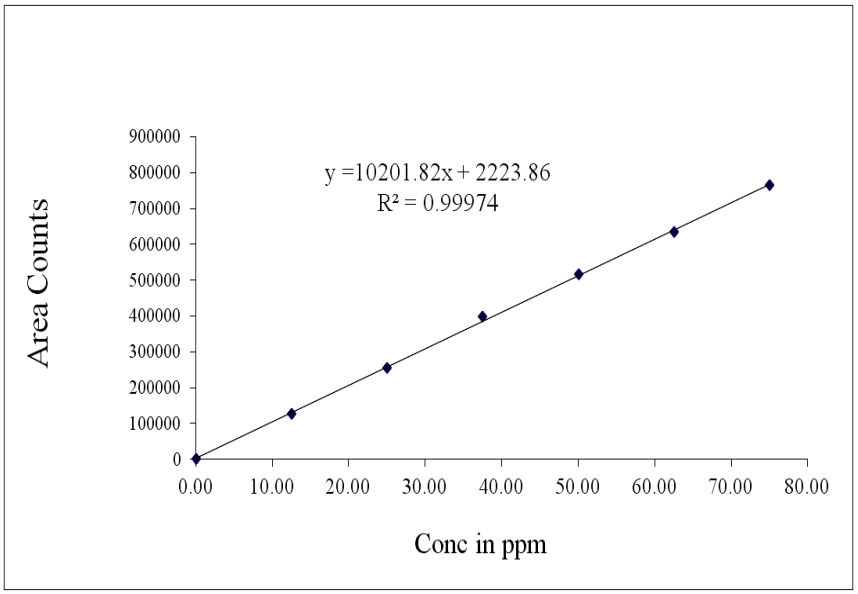

Fig No.12: calibration curve for Vildagliptin at 235 nm

Conclusion:

A sensitive, rapid, suitable, precise, linear, accurate and robust UPLC method has been developed for the determination of Dapagliflozin, Metformin and Vildagliptin impurities in pharmaceutical tablets. All the impurities were well resolved. All parameters meet the acceptance criteria for method validation according to the FDA and ICH specifications and method shows suitability, precision, accuracy, specificity, linearity and robustness. This developed method was provided better applications inter of robustness and filter compatibility studies than the literature reports. The developed method can be applied for routine analysis to quantify the process as well as degradation impurities in quality control laboratories.

References:

1. Collier, Roger (2012). "Reducing the "pill burden"". Canadian Medical Association Journal. **184** (2): E117–E118. doi:10.1503/cmaj.109-4076.
2. "5-in-1 PolyPill Treatment May Prevent Heart Disease" 2014-02-27 at the Way back Machine, BAYVIEW PHARMACY'S PRESCRIPTION COMPOUNDING BLOG,Apr 01, 2009 @ 08:09 AM.
3. Combination Products-U.S. Food and Drug Administration.

4. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. Foretz M, Guigas B, Viollet B. Nat Rev Endocrinol. 2019;15:569–589.
5. Combination therapy for type 2 diabetes: dapagliflozin plus metformin. Tan X, Hu J. Expert Opin Pharmacother. 2016;17:117–126.
6. Combination therapy with metformin plus vildagliptin in type 2 diabetes mellitus. Guarino E, Nigi L, Patti A, Fondelli C, Dotta F. Expert Opin Pharmacother. 2012;13:1377–1384.
7. Different glucagon effects during DPP-4 inhibition versus SGLT-2 inhibition in metformin-treated type 2 diabetes patients. Alsalim W, Persson M, Ahrén B. Diabetes Obes Metab. 2018;20:1652–1658.
8. Comparison of efficacy between dipeptidyl peptidase-4 inhibitor and sodium-glucose cotransporter 2 inhibitor on metabolic risk factors in Japanese patients with type 2 diabetes mellitus: Results from the CANTABILE study. Son C, Makino H, Kasahara M, et al. Diabetes Res Clin Pract. 2021;180:109037.
9. A network meta-analysis for efficacy and safety of seven regimens in the treatment of type II diabetes. Wang LG, Wang H, Liu Q, Hua WC, Li CM. Biomed Pharmacother. 2017;92:707–719.