WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Development and Validation of New Analytical Method for the Simultaneous Estimation of Dapagliflozin and Vildagliptin in Pharmaceutical Dosage Form

[1]Sai Dhanraj ,[2]Dr ShobhaRani.

[1]Post Graduate Student,[2]Professor & Head
[1]Pharmaceutical Analysis
[1]Centre of pharmaceutical sciences IST JNTU-H HyderabadI

ABSTRACT

on the basis of the combined calculation of the tablet dose forms of Vildagliptin and Dapagliflozin. The chromatogram was generated using a Kromasil (250mm 4.6mm, 5µ) column. A mobile phase was introduced into the column at a flow rate of 1.0 ml/min, consisting of OPA, acetonitrile, and methanol at a ratio of 48:52. The temperature was kept at 30 degrees Celsius. A wavelength of 215 nm was shown to be optimal for both Vildagliptin and Dapagliflozin. The Rt of Vildagliptin was 2.430 minutes, whereas that of dapagliflozin was 2.821 minutes. 0.6 and 0.4 were determined to be the %RSD of Vildagliptin and Dapagliflozin, respectively. %The recovery rates for Vildagliptin and Dapagliflozin were 99.70% and 99.71%, respectively. The values for the limits of detection (LOD) and quantification (LOQ) were 0.12 and 0.36, respectively, derived from the Vildagliptin and Dapagliflozin regression models. The Vildagliptin regression equation is y = 14257x + 32709, and the Dapagliflozin regression equation is y = 3242.3x + 1258.2.

Key Words: Vildagliptin and Dapagliflozin RP-HPLC

1. Introduction

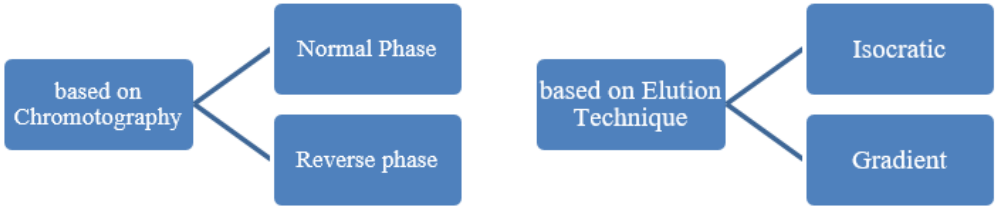
The effectiveness and safety of medicines are directly impacted by their quality. The well-being of patients depends on rigorous quality assurance and control procedures for chemical and pharmaceutical formulations. Therefore, in order to ascertain whether a medicine is suitable for a patient, it is necessary to investigate its pharmacological dose forms and individual medicinal components. Data analysis is only as good as the process used to collect it. Consequently, regulatory agencies require trustworthy and strong analytical methods to verify the efficacy of medications and their dose forms. The purity and safety of pharmaceuticals are often guaranteed by meticulous testing and impurity control. The pharmaceutical efficacy is determined by thorough testing, while the safety is shown by contaminants.

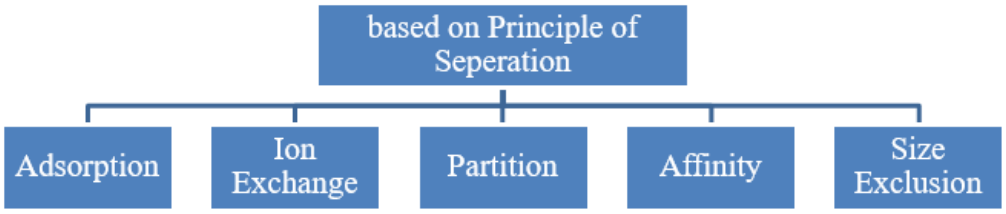
Chromatography

Chromatography is a general term for a variety of scientific techniques used to separate compounds. By passing a solution submerged in a "mobile phase" through a stationary phase, additional molecules can be separated from the analyte for analysis.

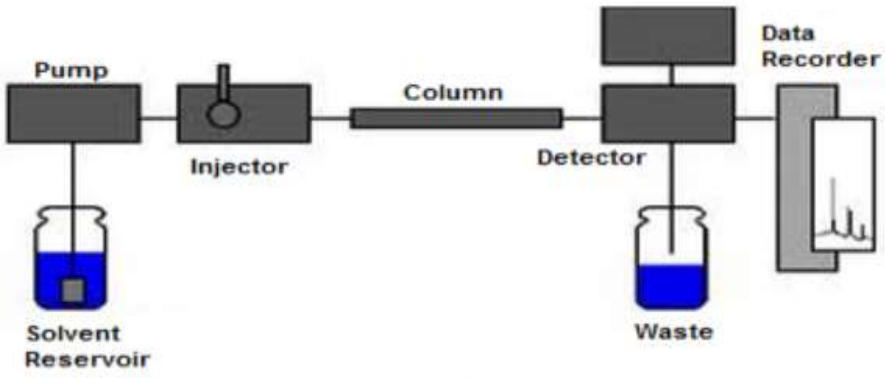
Using the analyte's high attraction to adsorbent particles, the separation is successfully done. Partitioning and selectively absorbing the analyte from a solution containing solid particles allows for elution; subsequent passage through a combination of liquid solvents completes the process.

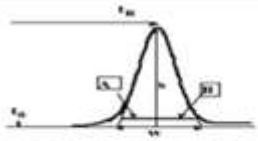
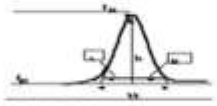
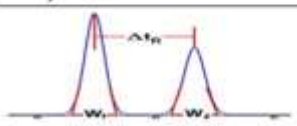
High Performance Liquid Chromatography Classification:





Instrumentation of Rp-HPLC



Parameter	Formula
Capacity Factor(K')	$K' = (t_R - t_M) / t_M$ Where: t_R= duration of the solute's retention t_M= duration of unretained solute retention
Tailing Factor (Tf)	 T = Total peak width / (2 x front half-width).
Column Efficiency (N)	 N = 5.54 (tR/ W) 2 = L / H
Resolution (Rs)	 RS = (tR2-tR1) / 0.5(w1+w2) Where: tR1 and tR2 = Their retention durations are shown. w1 and w2 = show two compound widths.
Retention time (Rt)	Rv = Rt x elution rate

Parameters Limit

Parameter	Limit
Capacity Factor	The void volume is typically K>2, and the peak should be clearly separated from neighboring peaks.
Tailing Factor	T ≤ 2
Theoretical plates	> 2000
Resolution	Rs of > 2 between the peak of interest and the closest eluting
Repeatability	≤ 2%

HPLC Method Validation:

Giving evidence that has been independently confirmed with a high level of certainty regarding the correctness of a certain attribute. A tried-and-true way for exhibiting the perceptual approaches that work and are practically applicable. One of the most common methods for determining if a research strategy is appropriate for its stated goal is the concept of strategy validity.

Method Validation Characteristics:

Characteristics	Function
Accuracy	It is close ties between the genuine value and the reference for estimating drug recovery.
Precision	uniform sample introduction multiple times to observe the degree of repeatability
Limit of Detection	The minimum amount of a material that has statistical significance in its detection.
Limit of Quantification	The lowest amount of a material that a control sample can contain and nonetheless be reasonably accurate and repeatable
Robustness	Method was carefully altered to enable elution in the specified manner and pass the RSD.
Linearity	Obtain the test results within the specified concentration range, from lower to upper limits.
Specificity	There is evidence of the primary component interfering with outside variables.
sensitivity	To determine which are low conc and measure them using LOD/Q
Stability	To determine how a material degrades under different stress situations

The Boundaries of Features:

Characteristics	Acceptance Criteria
Accuracy/trueness	Recovery 98-102% (individual)
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity / Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10
Linearity	Correlation coefficient $R^2 > 0.999$
Range	80 –120 %

Method Development for HPLC:

In line with the agency's rules, several chromatography methods and columns have been tested, as the analyte seems to be better analysed using less complex techniques. The HPLC method involved a number of steps to find the chemicals.

2. Drug Profile Vildagliptin and Dapagliflozin:

2.1 **Vildagliptin**

Description: Vildagliptin is a once-daily dipeptidyl peptidase 4 (DPP-4) inhibitor used in the management of type 2 diabetes mellitus.

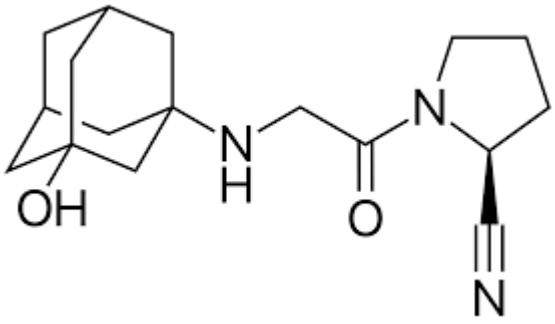


Figure 1: Structure of Vildagliptin

CAS Number	:	274901-16-5
IUPAC Name yl)amino]acetyl}pyrrolidine-2-carbonitrile	:	(2S)-1-{2-[(3-hydroxyadamantan-1-
Molecular Weight Monoisotopic	: :	Average: 303.3993 303.194677059
Molecular Formula	:	$C_{17}H_{25}N_3O_2$
Appearance	:	powder
Physical State	:	solid
Solubility	:	water solubility (1.75 mg/mL)
Log p	:	1.12
pK Values	:	pKa: 14.71 (Acidic), 9.03 (Basic).

MOA: Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are incretin hormones that regulate blood glucose levels and maintain glucose homeostasis.

Absorption: 1.7 hrs

Metabolism: About 69% of orally administered vildagpliptin is eliminated via metabolism not mediated by cytochrome P450 enzymes.

2.2 Dapagliflozin

Description: Dapagliflozin is a sodium-glucose cotransporter 2 inhibitor used in the management of type 2 diabetes mellitus.

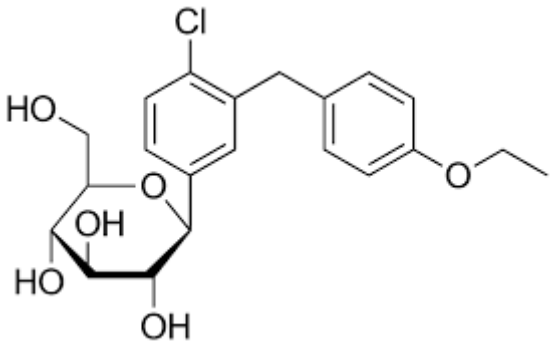


Figure 2: structure of Dapagliflozi

CAS Number	:	461432-26-8
IUPAC Name	:	(2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol
Molecular Weight	:	Average: 408.873
Monoisotopic	:	408.133966239
Molecular Formula	:	$C_{21}H_{25}ClO_6$
Appearance	:	Powder
Physical State	:	Solid
Solubility	:	water soluble (0.173 mg/mL)
Melting Point (°C)	:	65
Boiling Point (°C)	:	609
Log p	:	2.7
pK Values	:	pKa: 12.57 (Acidic), -3 (Basic).

MOA: inhibits the sodium-glucose cotransporter 2(SGLT2) which is primarily located in the proximal tubule of the nephron.

Absorption: 1 hrs

Metabolism: Dapagliflozin is primarily glucuronidated to become the inactive 3-O-glucuronide metabolite(60.7%)

Aim, Purpose

Aim:

This Project aims to develop a quick, accurate, incredibly responsive, dependable and secure analytical technique for evaluating hard and tablet Vildagliptin and Dapagliflozin.

Objective:

Picking an appropriate detection mechanism, mobility phase, data column to analyse, flow rate, temperature, and pH concentration; and deciding on an HPLC separation chemical.

Components and Procedures

Pharmaceutical Industry supplied us with the necessary active pharmaceutical ingredient (API) gift sample, which is utilised in this approach. The components employed are of high quality or grade 1.

Combination Vildagliptin and Dapagliflozin tablet Nextstellis supplied from Wellness Pharma international is Obtained from Market.

Other Chemicals and Solvents used are:

Water	0.1 - 2 N HCl
MeCN	H_2O_2
Trimethyl Amine	NaOH
KH_2PO_4	OPA

All the above chemicals and solvent

Sample Processing

Diluent: MeCN and H20 are combined at an 80:20 ratio.

Validation:

System suitability parameters:

In order to establish system parameters, we employed Vildagliptin (20 ppm) and Dapagliflozin (20 ppm) reference solutions. We injected the solutions six times and measured the peak melting point, and plate count.

Specificity: Analysing the effect of optimal procedures by empirical means. In this method, the blank and placebo samples should not show a peak following the medication delivery. A straightforward methodology was described

Precision:

sample working sols (100% sol) From stock sol 1 ml of the sol was added to a 10 ml vf and makeup with diluent. (10µg/ml Vildagliptin, 100µg/ml Dapagliflozin)

Linearity:

From stock sol following volumes are pipetted out in different vol flasks.

Level	ml
25 %	0.25 ml from stock
50 %	0.5 ml from stock
75 %	0.75 ml from stock
100 %	1.0 ml from stock
125 %	1.25 ml from stock
150 %	1.5 ml from stock

Accuracy:

50% sol	0.5 ml of sample stock and 1 ml stock added to 10ml and makeup witl dil
100% sol	1 ml of sample stock and 1 ml stock added to 10ml and makeup witl dil
150% sol	1.5 ml of sample stock and 1 ml stock added to 10ml and makeup witl dil

All levels must have a recovery rate between 98% and 102% to be eligible.

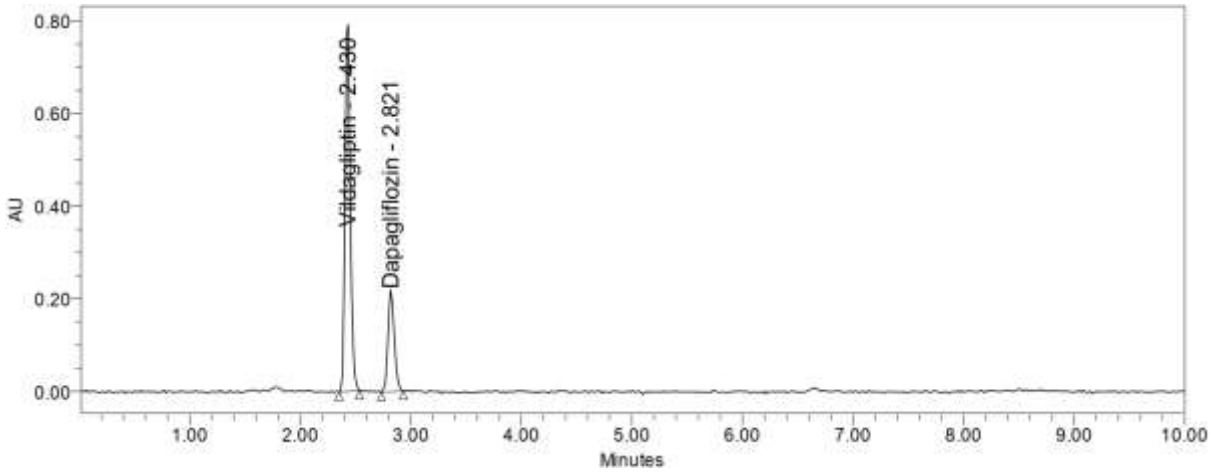
LOD and LOQ prep: 0.31 ml of stocl sol was added in 2 different 10ml vf and filled with dil

LO-D	From above sol 0.1 ml of each stock taken in 10ml vf and makeup wil dil
LO-Q	0.3 ml of each stock in 10ml vf and makeup with dil

Flow minus	0.9 ml/min
Flow Plus	1.1 ml/min
Temp -	22°C
Temp +	31°C
Mobile phase-	
Mobile phase+	

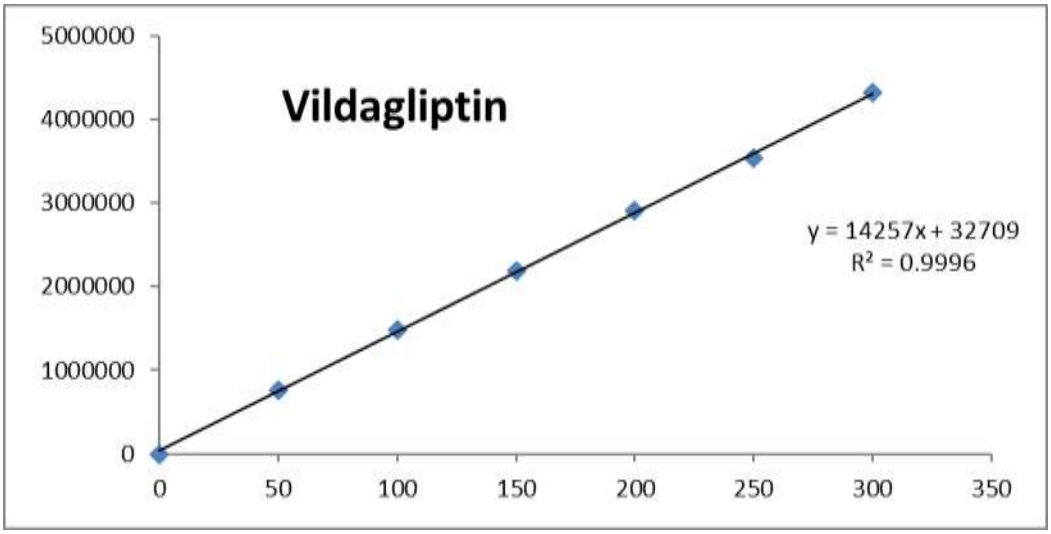
Optimized Chromatogram

Movable phase	OPA: Acetonitrile (48:52)
Column used	Kromasil (2.1 x 150mm, 1.7µm)
Stream rate	1.0 ml/min
λ max	215
C Temp	30°C
Inj	10 µL
Range	10 min

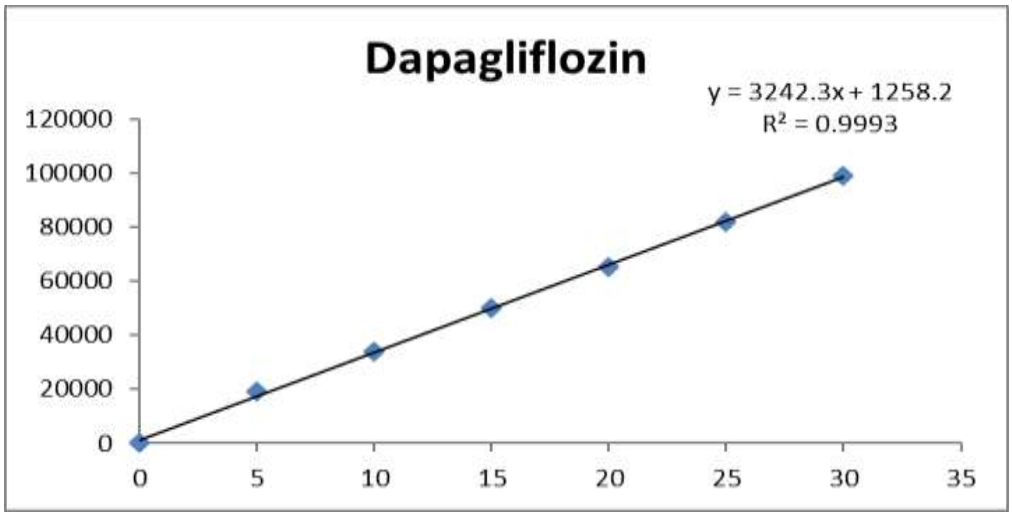


Structure of optimized Chromatogram

Remark: With high resolution,Optimization and Validation of this method was achieved as Vildagliptin and Dapagliflozin separated at 2.543 and 3.153 minutes. Excellent plate count and



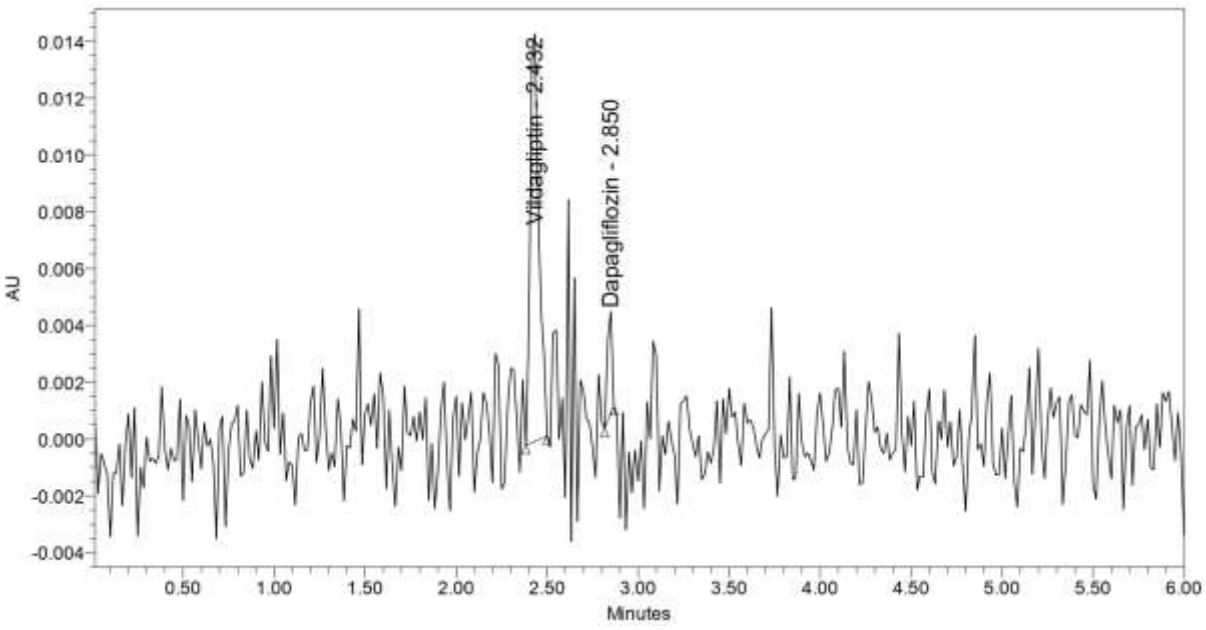
Calibration Data Of Vi



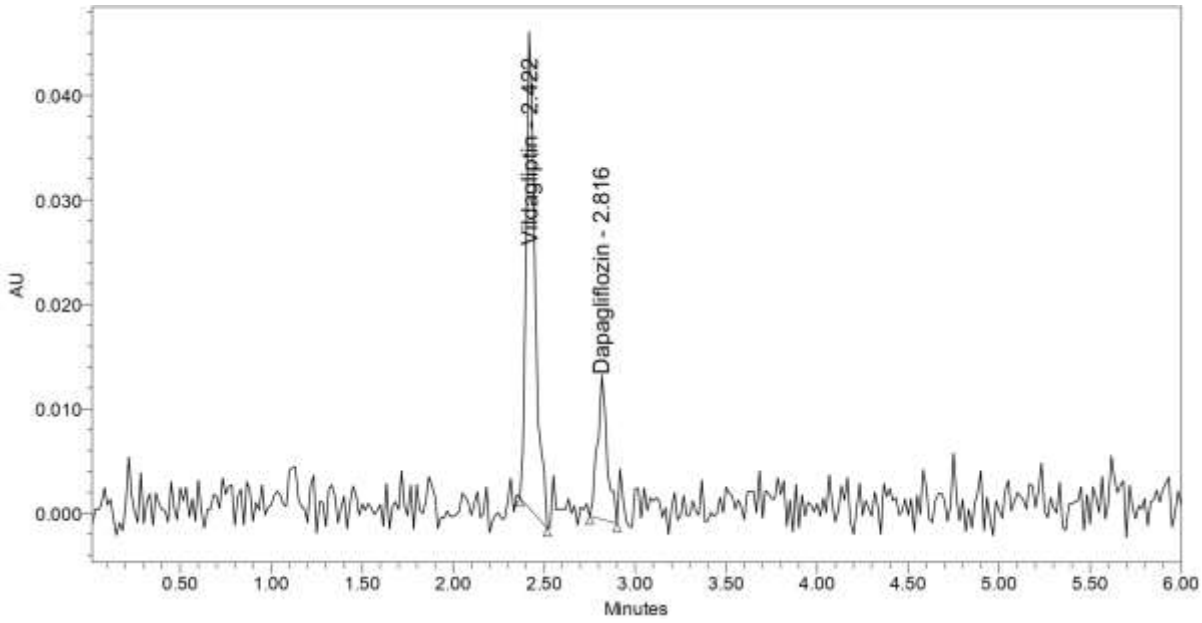
Calibration Data Of Dapagliflozin

Remark:

	Vildagliptin	**Dapagliflozin**
Concentration range (µg/ml)	50-300	5-30
Regression Equation	y = 14257x + 32709	y = 3242.3x + 1258.2
Co-relation	0.999	0.999

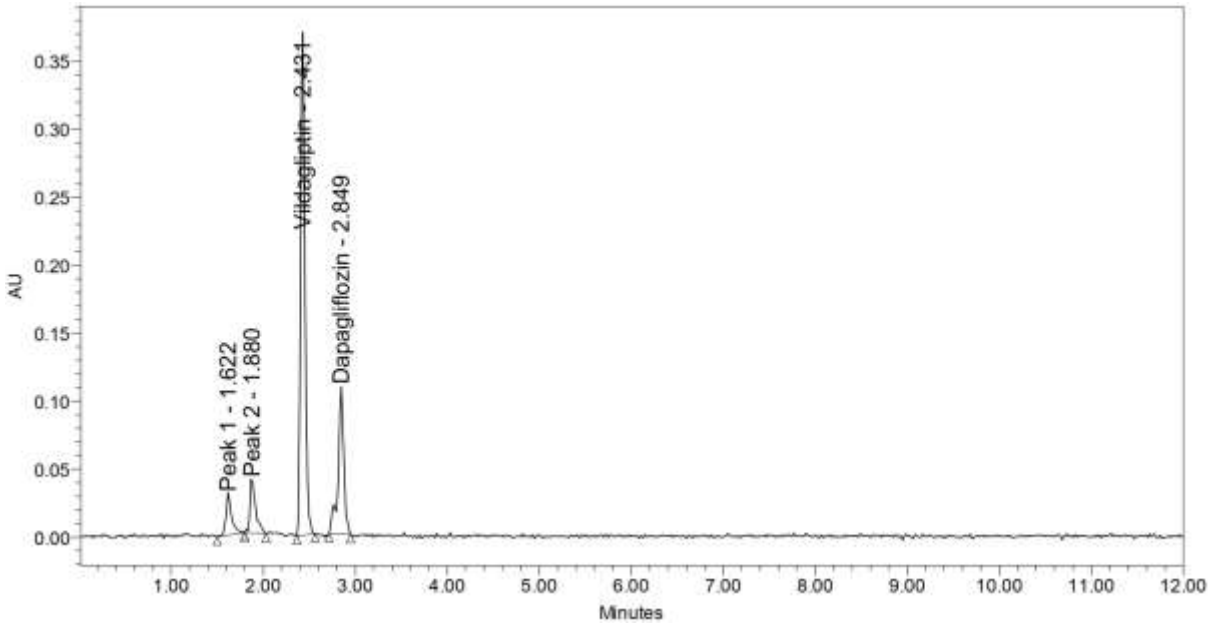


LOD Chromatogram

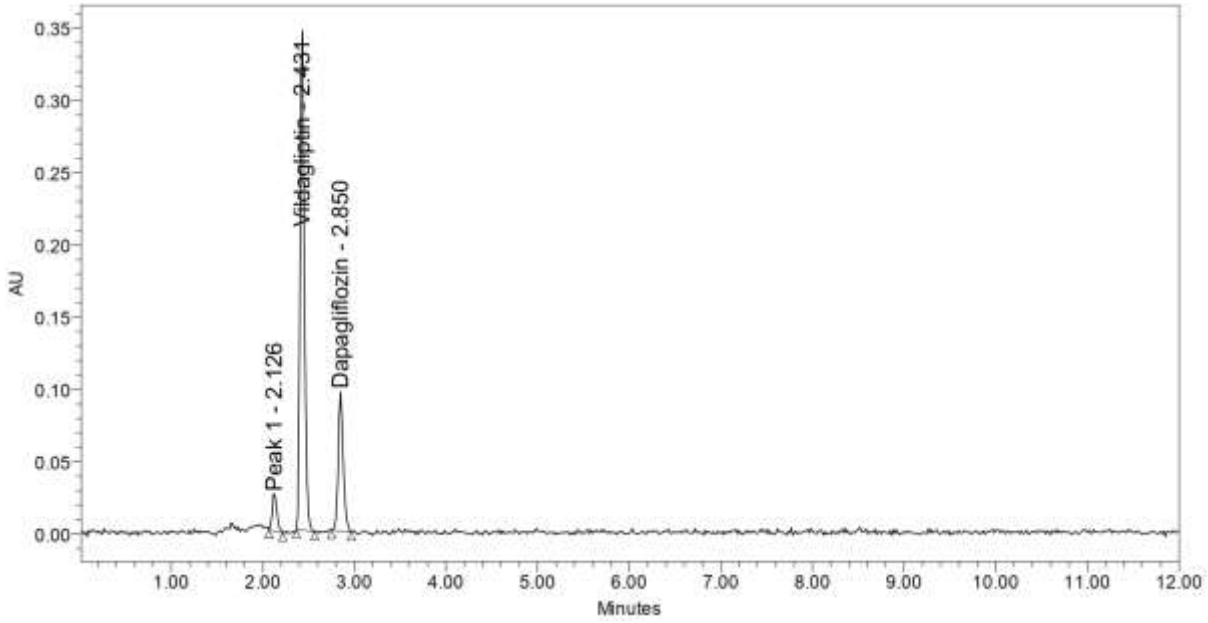


LOQ Chromatogram

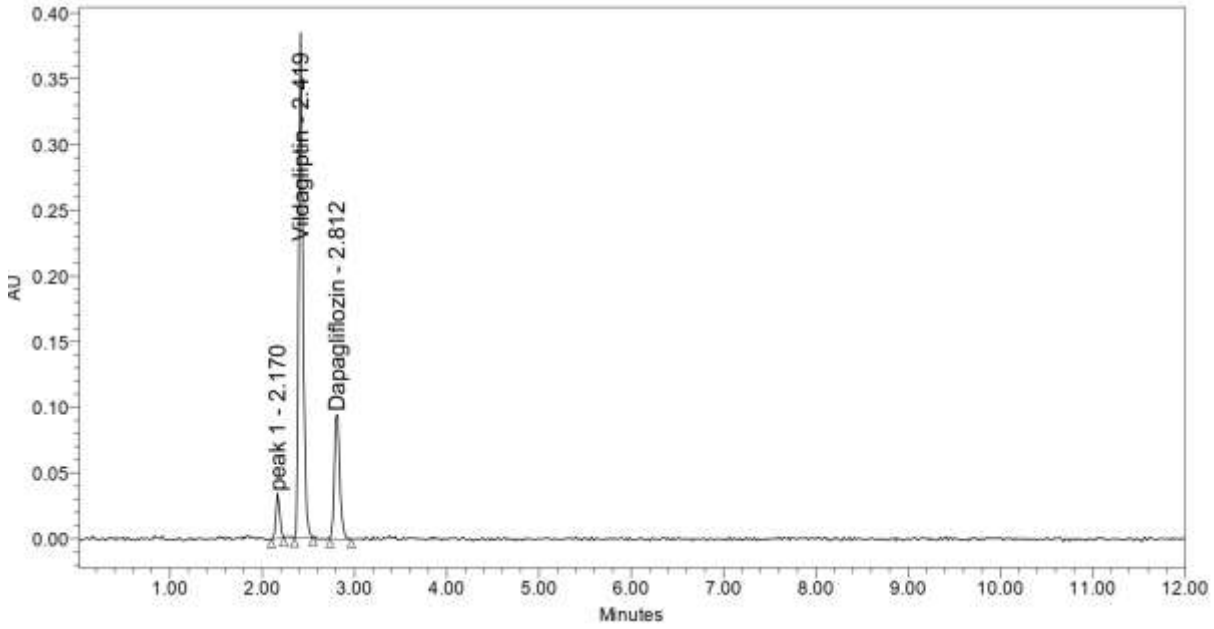
Remark: According to ICH guidelines all parameters were passed


AU
Peak 1 - 1.622
Peak 2 - 1.880
Vildagliptin - 2.431
Dapagliflozin - 2.849
Minutes

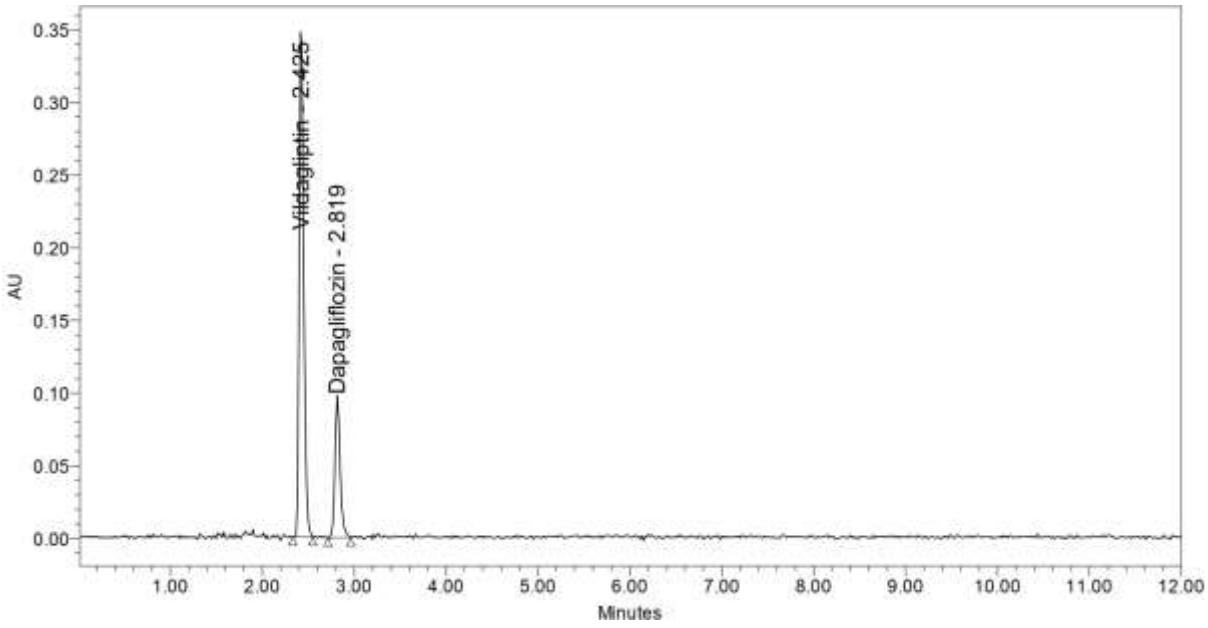
Acid

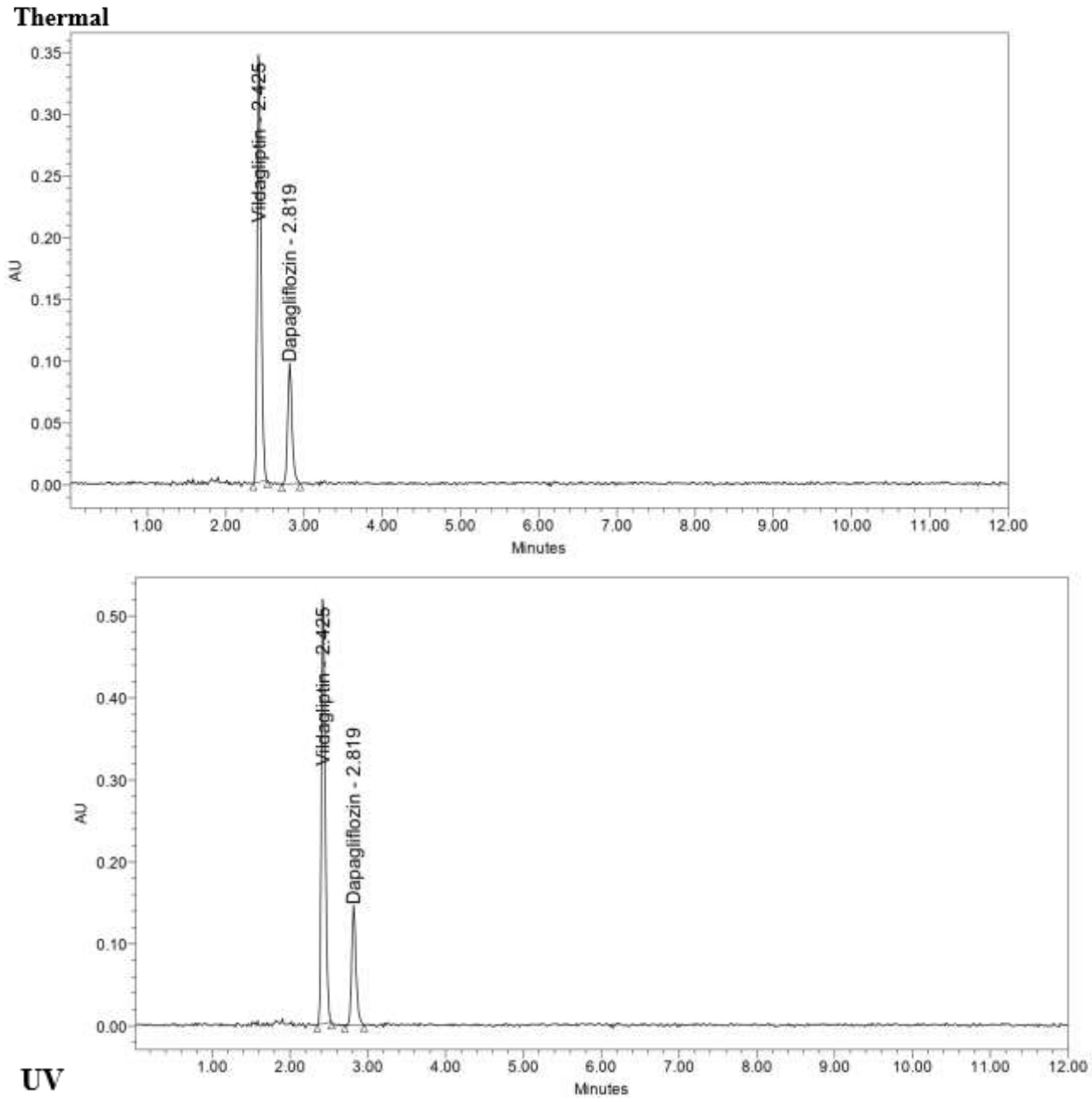

AU
Peak 1 - 2.126
Vildagliptin - 2.431
Dapagliflozin - 2.850
Minutes

base


AU
Minutes
peak 1 - 2.170
Vildagliptin - 2.419
Dapagliflozin - 2.812

Peroxide


AU
Minutes
Vildagliptin - 2.425
Dapagliflozin - 2.819



Conclusion:

The simultaneous measurement of both pharmaceuticals using HPLC is a reliable, accurate, and efficient analytical method for quantifying dapagliflozin and vildagliptin in pharmaceutical dosage forms and bulk. Since the authorized approach clearly and sharply separates the two compounds, it quantifies them precisely without interference from degradation products or excipients. The chromatographic parameters, encompassing the mobile phase composition, flow rate, and detection wavelength, have been meticulously adjusted to provide superior sensitivity, specificity, and repeatability. Techniques that meet ICH requirements for linearity, precision, accuracy, and durability can be applied in both industrial and medical contexts.