International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Development and Validation of Stability Indicating Rp-Hplc Method for Estimation of Teneligliptin, Dapagliflozin and Metformin in Combined Dosage Form

Dr. David Adjei

Lecturer (Valley View University, Kumasi School of Education (Social Studies) Valley View University Kumasi Campus-Ghana

Abstract:
This study presents the development and validation of a simple, specific, accurate, and precise stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Teneligliptin, Dapagliflozin, and Metformin in a combined dosage form. The separation was achieved on an Inertsil C-18, 4.6 mm x 250 mm column with isocratic flow. The mobile phase at a flow rate of 1.0 ml/min consisted of Methanol and Buffer (70:30, v/v). The UV detection was carried out at 254nm. Teneligliptin retained 6.01 min, Dapagliflozin retained 7.65 min and Metformin retained at 3.85. Teneligliptin revealed a linear response in the concentration range of 2-20ppm. In the concentration range of 1-10ppm dapagliflozin demonstrated a linear response, while metformin revealed a linear response in the concentration range of 50-500ppm. Correlation coefficients ('R' value) was found to be 0.9912 for Teneligliptin, 0.9983 for Dapagliflozin and 0.9914 for metformin. The % recovery was found to be 99.9% for Teneligliptin, 99.5% for Dapagliflozin and 99.6% for metformin. Limit of detection and limit of quantitation were found to be 0.05µg/ml & 0.18µg/ml for Teneligliptin, 0.04 & 0.12 µg/ml for Dapagliflozin and 0.56 & 1.72 µg/ml for Metformin. The method was successfully validated as per ICH guidelines and demonstrated to be suitable for routine quality control and stability assessment.

INTRODUCTION:

Teneligliptin is a DPP-4 inhibitor, Dapagliflozin is an SGLT2 inhibitor, and Metformin is a biguanide, all of which are oral antidiabetic agents used in managing type 2 diabetes mellitus. Type 2 diabetes mellitus is a chronic condition where high blood sugar levels occurs due to either the body not producing enough insulin or the cells not responding properly to insulin (insulin resistance). These three drugs, when used in combination, show a synergistic effect to control blood glucose levels. Thus, a validated, stability-indicating RP-HPLC method for their simultaneous estimation in pharmaceutical dosage forms is essential.

High-performance liquid chromatography (HPLC) is one of the most widely used analytical techniques due to its high sensitivity, precision, and ability to analyze multiple components in a single run. The reverse-phase mode (RP-HPLC) utilizes a non-polar stationary phase and a polar mobile phase, making it particularly effective for separating polar compounds like antidiabetic agents. A stability-indicating

method is a validated analytical procedure that accurately measures active ingredients free from interference from degradation products, process impurities, and excipients. Such methods are indispensable in pharmaceutical development for determining the shelf life, quality control, and regulatory compliance. In reverse-phase HPLC, hydrophobic interactions dominate, allowing for effective separation of polar and semi-polar molecules like the antidiabetics in this study. Additionally, the use of a UV detector enables precise quantification due to the chromophoric properties of the compounds.

Teneligliptin is a recently developed oral dipeptidyl peptidase 4 inhibitor indicated for the management of type 2 diabetes mellitus (T2DM), Teneligliptin is chemically known as {(2S,4S)-4- [4- (3-Methyl -1-phenyl - 1H-pyrazol-5-yl) 1-piperazinyl]-2-pyrrolidinyl}(1,3-thiazolidin-3-yl) methadone, C22H30N6OS with molecular weight 426.6 gm/mol.
Teneligliptin is a DPP-4 inhibitor, a class of drugs used to treat type 2 diabetes mellitus. It works by blocking the enzyme dipeptidyl peptidase-4 (DPP-4), which breaks down incretin hormones like GLP-1 and GIP. By inhibiting DPP-4, Teneligliptin increases the levels of these hormones, leading to increased insulin secretion and decreased glucagon secretion, which helps lower blood glucose levels.

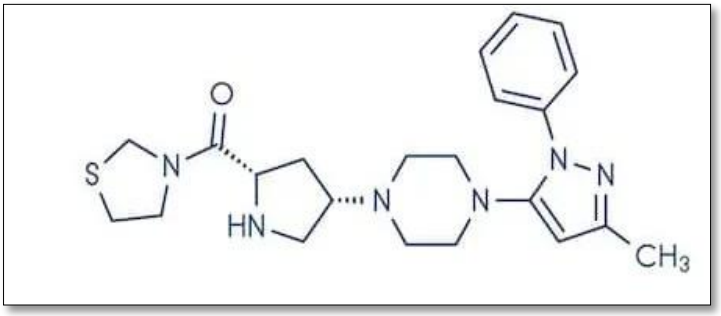

FIG. NO. 1: Chemical Structure Of Teneligliptin

Dapagliflozin, Dapagliflozin was the first drug in a class of therapies for glycaemic control in adults with type 2 diabetes (T2D). The chemical name for dapagliflozin is (2S,3R,4R,5S,6R)-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol. It is also known as BMS-512148 or (1S)-1,5-anhydro-1-C-{4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-D-glucitol. It is an inhibitor of the sodium glucose cotransporter, resident in the proximal nephron, which is responsible for the recovery of filtered glucose back into circulation. Inhibiting this cotransporter reduces glucose recovery, increases glucose excretion, and reduces hyperglycaemia.

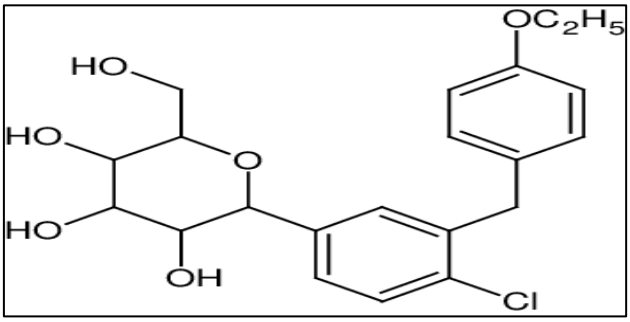

FIG. NO. 2: Chemical Structure Of Dapagliflozin

Metformin The chemical name for metformin is 1,1-Dimethylbiguanide hydrochloride. It is also

referred to as N,N-Dimethylimidodicarbonimidic diamide hydrochloride. It belongs to the biguanide class of antidiabetic drugs. It is the first line drug of choice for the treatment of type-2 diabetes. It activates adenosine monophosphate activated protein kinase, a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and metabolism of glucose and fats. Biguanides lower blood glucose levels by decreasing the amount of glucose your liver produces and releases into your bloodstream.

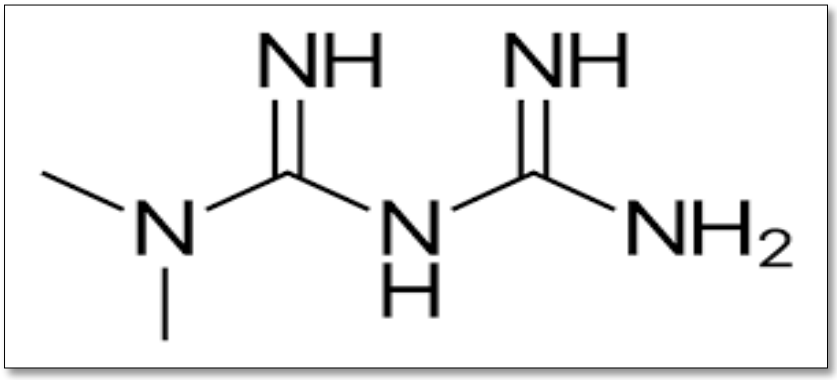

FIG. NO. 3: Chemical Structure Of Metformin

They also help lower blood glucose levels by making your skeletal muscle tissue more sensitive to insulin so it can absorb glucose for energy. This increases insulin sensitivity and reduces insulin resistance. These bodily changes help manage diabetes**.**

MATERIALS AND METHODS:

Instruments Employed:

HPLC: AGILENT INFINITY 1100
Digital Balance: Wenser
pH Meter: Digital pH meter Instrument india
Sonicator: Ultrawave, Instrument india
Membrane filter: Nylon Membrane filter (0.45)
Software used: ChemStation

Chemicals and Reagents:

Active Pharmaceutical Reagent used : Teneligliptin Purity of API is (99%), Dapagliflozin purity of API (99%) and Metformnin Hydrochloride of API (99%).

Pharmaceutical Formulation : Tablets of Teneligliptin, Dapagliflozin and Metformin with strength 10 mg, 20mg and 500mg Brand name as ZITA-DM manufactured by Glenmark were used. These tablets were purchased from local pharmacy.

Reagents and Chemicals used: Formic acid and Milli-Qwater (HPLC Grade), Potassium dihydrogen Phosphate, Orthophosphoric Acid were used for preparing mobile phase.

Preparation of Mobile Phase: 70 volumes of HPLC grade Methanol and 30 volumes of Buffer was used as mobile phase.

Preparation of Buffer Phase: 1.0% v/v formic acid in water (Take 10 ml of formic acid with 500 ml water and make up volume 1000 ml with water).

Preparation of Diluent: (Methanol:Water) (70:30) was chosen as a diluent based on the solubility of the medication

Preparation of Standard Solution: 20mg Teneligliptin, 10mg Dapagliflozin and 500 mg metformin working standard were accurately weighed transferred into10 mL volumetric flask respectively. About 4ml diluent was added, sonicated to dissolve and diluted to 10mL using diluent. Accurately 1mL of Teneligliptin 1mL of dapagliflozin and 1 ml of metformin solutions were transferred in to 10mL volume tri flask, Volume was made up with diluents to get 20ppm of Teneligliptin 10ppm of Dapagliflozin and 500 ppm of metformin.

Preparation Sample solution : Twenty tablets were weighed and finely powdered. An accurately weighed tablet powder equivalent to 50.0 mg of METF, 2.0 mg of TEN and 1.0 mg of DAPA (120.69 mg) was transferred into a 10.0 ml volumetric flask. About 5.0 ml of methanol was added and mixture was sonicated for 10 min. The solution was cooled to room temperature and diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper and 1 ml was transferred to a 10.0 ml volumetric flask and then volume was made up to the mark with mobile phase to obtain a concentration of 500 µg/ml, 20 µg/ml and 10 µg/ml of METF, TEN and DAPA respectively.

Method Development : Chromatographic separation was accomplished using a Inertsil C-18, 4.6 mm x 250 mm column with methanol and buffer in the ratio of (70:30) as the mobile phase at a flow rate of 1 mL/min and column temperature of 30°C, with detection at 254 nm. The optimized approach resulted in an elution time of 6.01 min for Teneligliptin, 7.65 min for Dapagliflozin and 3.85 min for metformin. The overall running time was 10 min. Fig. 4 exhibit chromatograms of Teneligliptin, Dapagliflozin and Metformin.

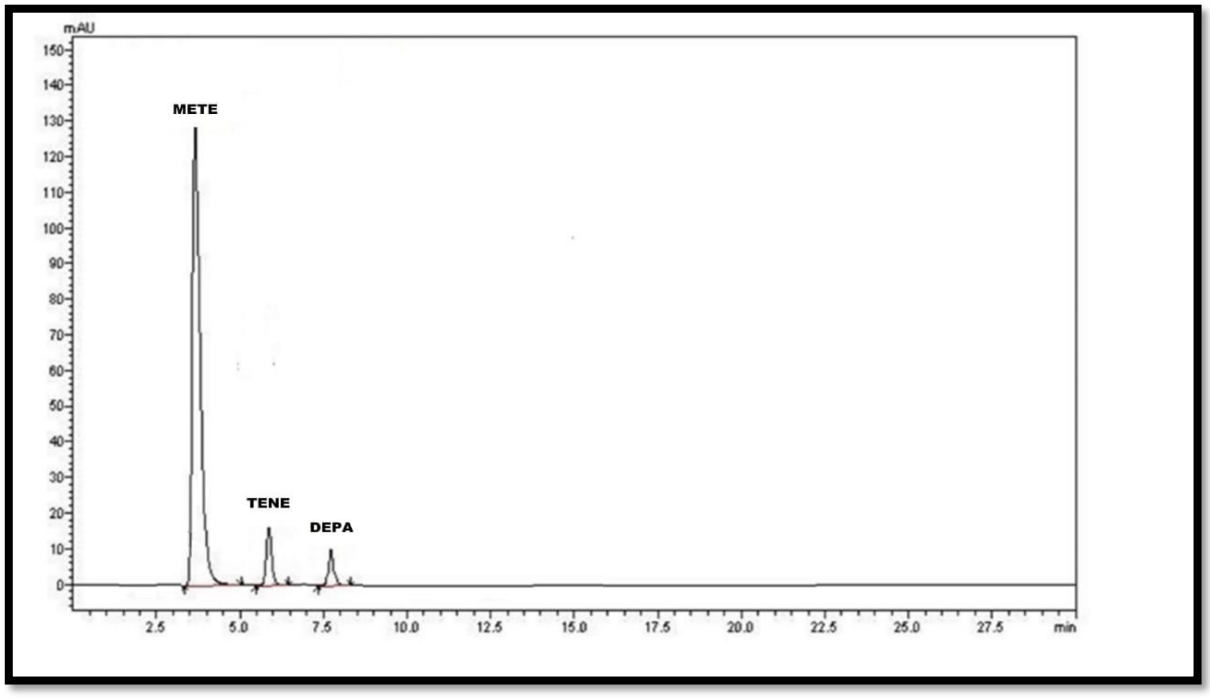

FIG. NO. 3: Chromatogram Of Teneligliptin, Dapagliflozin and Metformin

Optimized Chromatographic conditions:

The following chromatographic parameters were established on trial and error basis and were kept constant during experimentation.

Column: Inertsil C-18, 4.6 mm x 250 mm

Detection wavelength: 254 nm

Injection volume: 20 µl
Runtime: 10 minutes
Flow rate: 1ml/ min
Temperature: 30° C
Mobile phase: Methanol and Buffer (70/30, v/v)
Mode of Operation: Reverse phase

RESULT AND DISCUSSION:

The developed RP-HPLC method for Teneligliptin, Dapagliflozin and Metfromin was validated as per ICH guidelines.

Specificity : Specificity is defined as the capacity to evaluate an analyte definitively in the presence of predicted components. The specificity studies were carried out by attempting deliberate degradation of the tablet sample with exposure to stress conditions like acidic, alkaline, neutral, oxidation, heat and sun light and it was found that drugs are stable at all the conditions.

Precision: Precision is the degree of closeness of agreement between the series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. It is expressed in terms of standard deviation (SD) or relative SD (RSD). Precision may be a measure of either the degree of repeatability or the reproducibility of the analytical method.

Validation parameters		**METF**			**TEN**			**DAPA**		
		Mean	**SD(±)**	**RSD(%)**	**Mean**	**SD(±)**	**RSD(%)**	**Mean**	**SD(±)**	**RSD(%)**
System Precision[a)]		3061172	4553.76	0.149	460290	111.07	0.024	350410	123.89	0.035
Method Precision[b)]		100.01	0.8969	0.8987	100.27	0.1162	0.1291	100.47	0.1490	0.1502
Intermedi -ate Precision	Intraday [b)]	99.98	0.6655	0.6656	100.32	0.1628	0.1629	100.54	0.0605	0.0608
	Interday [b)]	99.87	0.6458	0.6449	100.20	0.1265	0.1268	100.22	0.0642	0.0649
	Different Analyst [b)]	100.01	0.8120	0.8245	101.27	0.3512	0.3452	102.05	0.0845	0.0852

Table No. 1 : The results of system, method and intermediate precision

Linearity of response: Aliquoted portions of standard solutions, Metformin, Teneligliptin and Dapagliflozin (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml) were diluted to 10.0 ml with mobile phase to get concentration from 50, 100, 200, 300, 400, 500 µg/ml of METF and 2, 4, 8, 12, 16, 20 µg/ml of TEN and 1, 2, 4, 6, 8, 10 µg/ml of DAPA.. The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase to get the steady baseline.

Drug	Range (µg/mL)	Regression Equation	R^2
Teneligliptin	2–20	y = 14846x + 163639	0.9912
Dapagliflozin	1-10	y = 24788x + 105948	0.9983
Metformin	50-500	y = 5190.6x + 496328	0.9914

Table No. 2: Linearity Data

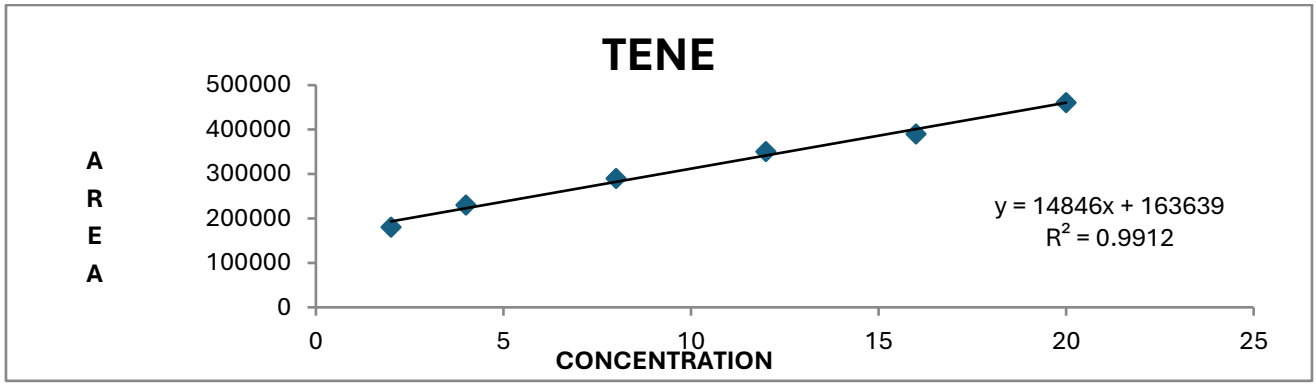

Fig No. 4: Calibration Curve Of Teneligliptin

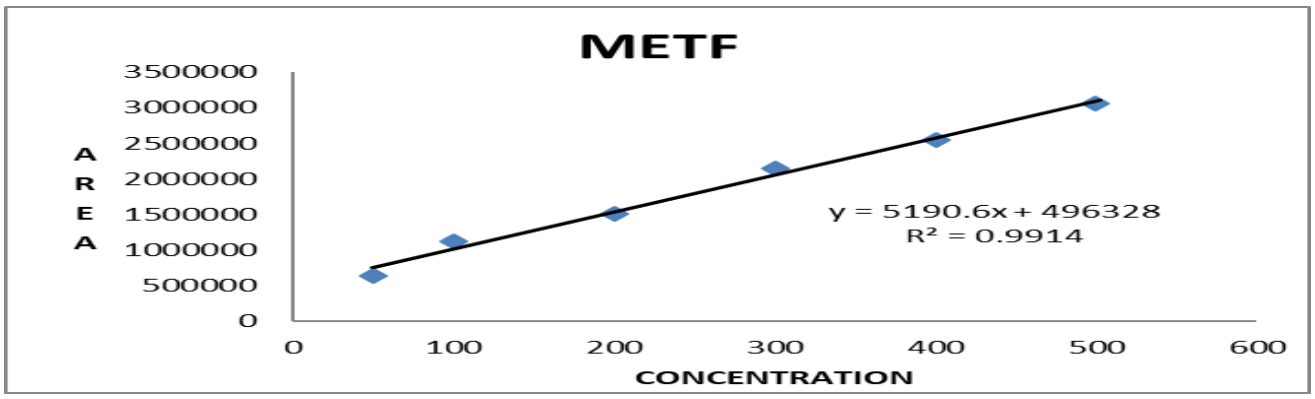

Fig No. 5: Calibration Curve Of Metformin

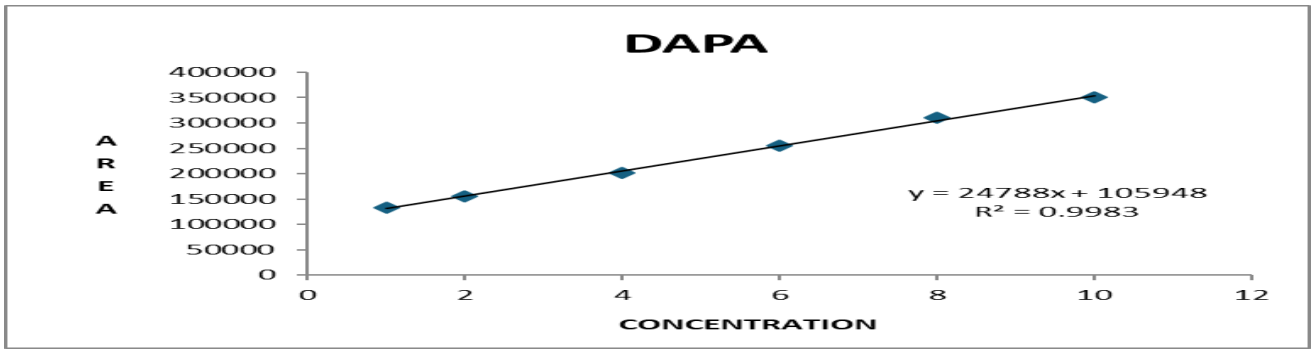

Fig No. 6: Calibration Curve Of Dapagliflozin

System Suitability Parameters:

Drug	Retention Time (min)	Tailing Factor	Theoretical Plates
Teneligliptin	6.01	1.33	3173

Dapagliflozin	7.65	1.22	5464
Metformin	3.85	1.06	6783

Table No. 3: System Suitability Parameters

Limit of detection (LOD) Limit of quantitation (LOQ)

LOD and LOO determination were done with method based on standard deviation of the response and the slope of calibration curve.

Parameters	**METF**	**TEN**	**DAPA**
Linear dynamic range (µg/ml)	50-500	2-20	1-10
Slope	5190.6	14846	24788
Correlation coefficient	0.9914	0.9912	0.9983
LOD (µg/ml)	0.56	0.05	0.04
LOQ (µg/ml)	1.72	0.18	0.12

Table No. 4: LOD & LOQ

Accuracy: The accuracy of the method was assessed by recovery experiments by adding a known quantity of pure standard drug to the sample solution and recovering the same in terms of its peak areas. The sample was spiked with standard at levels of 50%, 100%, and 150% of test concentrations.

Pre Analyzed Sample	**Level**	**Amount Added (mg)**	**Amount Recovered (mg)**	**% Recovered**	**Mean of % Recovered**
Teneligliptin	50%	0.8	0.95	100%	99.9%
	100%	1.0	0.99	100%	
	150%	1.2	1.01	99%	
Dapaglifllozin	50%	0.4	0.35	99%	99.5%
	100%	0.5	0.31	98%	
	150%	0.6	0.34	99%	
Metformin	50%	20.0	19.8	99.5%	99.6%
	100%	25.3	24.6	99.6%	
	150%	30.0	29.5	99.8%	

Table No. 5: Accuracy Data

Robustness: The robustness of the method was determined by making small deliberate changes in the method like flow rate, mobile phase ratio, and temperature.

Parameters	**-Level**	**Nominal**	**+Level**
Change in Scanning Wavelength(nm)	252nm	254nm	256nm
Change in methanol content in total mobile phase (ml)	68.0ml	70.0ml	72.0ml
Change in flow rate (ml)	0.8ml	1.0ml	1.2ml

Table No. 6: Parameters for robustness study

Conclusion:

The developed RP-HPLC method for the simultaneous estimation of Teneligliptin, Dapagliflozin, and

Metformin is precise, accurate, linear, and robust. It can effectively separate the analytes in the presence of degradation products, thus proving stability-indicating capability. The method is suitable for routine analysis and quality control in pharmaceutical formulations.

Reference:

1. Micheal E.Swartz, Ira S.Krull Analytical method development and validation 1997.25-83.
2. McClure, W.F. Analytical Chemistry, 1994, Vol. 66.
3. Warren Beach, Clinical Therapeutics, Vol. 20,1998.
4. National Institute for Health and Care Excellence (NICE). *Type 2 Diabetes: The Management of Type 2 Diabetes*. London: NICE; 2009. Available from: http://www.nice.org.uk/Guidance/CG87. Accessed August 18, 2014.
5. Garber AJ, Abrahamson MJ, Barzilay JI, et al. AACE comprehensive diabetes management algorithm 2013. *Endocr Pract*. 2013;19(2):327–336.
6. Harris, D. C. (2015). Quantitative Chemical Analysis (9th ed.). W.H. Freeman.
7. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). Principles of Instrumental Analysis (7th ed.). Cengage.
8. Suryawanshi, P., et al. (2021). "RP-HPLC Method development for estimation of Dapagliflozin." Research Journal of Pharmacy and Technology.
9. Cwynar-Zając, Ł. (2021). "Metformin – A new approach." Pharmaceutical Reports.
10. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2011). Introduction to Modern Liquid Chromatography (3rd ed.). Wiley.