Journal of Molecular Science

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

Journal of Molecular Science

www.jmolecularsci.com ISSN:1000-9035

Concurrent Assessment of Baxdrostat and Dapagliflozin by Validated Uv Spectrophotometric and RP-HPLC Techniques: Development And Statistical Comparison

Riya N. Shukla[1], Nusrat K. Shaikh[2], Foram A. Patel[3], Jitendra O. Bhangale[4]
[1]Student, Smt. N. M. Padalia Pharmacy College, Navapura, Ahmedabad, Gujarat, India 382210
[2]Associate Professor, Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat, 382210, India
[3]Assistant Professor, Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat, 382210, India
[4]Professor and Principal, Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat, 382210, India

Article Information
Received: 07-10-2025
Revised: 24-10-2025
Accepted: 17-11-2025
Published: 26-12-2025

Keywords
Baxdrostat, Dapagliflozin, UV method, HPLC Method, Statistical Comparison.

ABSTRACT

Chronic kidney disease (CKD) is a major global health burden and is strongly associated with hypertension and diabetes mellitus, which significantly contribute to renal dysfunction and disease progression. Baxdrostat, a novel aldosterone synthase inhibitor, and Dapagliflozin, a sodium glucose cotransporter-2 (SGLT-2) inhibitor, have demonstrated renal and cardiovascular benefits individually and in combination. However, no validated analytical method has been reported for the simultaneous estimation of Baxdrostat and Dapagliflozin in synthetic mixtures. The present study aimed to develop and validate simple, accurate, and reproducible UV-spectrophotometric and RP-HPLC methods for the simultaneous estimation of Baxdrostat and Dapagliflozin in accordance with ICH Q2 (R2) guideline. The UV method employed first-order derivative spectrophotometry using methanol as solvent, with measurements at zero-crossing points of 255 nm for Baxdrostat and 232 nm for Dapagliflozin. The RP-HPLC method utilized a Kromstar C_{18} column with a mobile phase consisting of 0.1% formic acid in acetonitrile and phosphate buffer (pH 3.4) in a 20:80 %v/v ratio, at a flow rate of 1.0 mL/min and detection at 232 nm. Both methods showed excellent linearity over the studied concentration ranges, with correlation coefficients greater than 0.999. Precision, accuracy, robustness, LOD, and LOQ were within acceptable limits, and assay results of the synthetic mixture were satisfactory. Statistical comparison using Student's t-test revealed no significant difference between the two methods. The developed methods are suitable for routine quality control analysis of Baxdrostat and Dapagliflozin in laboratory-prepared synthetic mixtures.

1. INTRODUCTION:

Chronic kidney disease (CKD) has emerged as a major global public health challenge, imposing a substantial burden on healthcare systems worldwide. According to the Global Burden of Disease (GBD) Study, approximately 697 million individuals were living with CKD globally in 2017, with a global prevalence of nearly 9.1% [1]. CKD was responsible for an estimated 1.2 million deaths directly and contributed significantly to cardiovascular mortality, ranking among the leading causes of death worldwide [1, 2]. The increasing prevalence of CKD over the past three decades is largely attributed to population aging and the growing incidence of non-communicable diseases. India bears a disproportionately high burden of CKD, reflecting the epidemiological transition

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

toward chronic metabolic disorders. GBD estimates indicate a substantial rise in CKD-related morbidity and mortality in India over the past two decades[3]. Community-based studies suggest that CKD prevalence in India ranges between 8-15% in the adult population, with impaired kidney function detected in approximately 3-5% of nationally representative samples [4, 5]. The rising burden of CKD in India parallels the rapid increase in hypertension (HTN) and diabetes mellitus (DM), which are recognized as the two most important modifiable risk factors for renal injury. Although standardized diagnostic criteria and improved screening strategies have enhanced early detection of CKD, prevention remains challenging. The growing burden of CKD, particularly among patients with coexisting hypertension and diabetes mellitus, necessitates the development of novel therapeutic strategies targeting multiple pathophysiological pathways. Baxdrostat (Figure 1-A), a selective aldosterone synthase inhibitor, has emerged as a promising agent for resistant hypertension by modulating mineralocorticoid-mediated renal and cardiovascular injury[6], while Dapagliflozin (Figure 1-B), a sodium glucose cotransporter-2 (SGLT2) inhibitor, has demonstrated significant renoprotective and cardioprotective benefits beyond glycemic control[7]. SGLT2 inhibitors are a class of prescription medications (including Dapagliflozin, Remogliflozin, Canagliflozin) that treat type 2 diabetes by forcing the kidneys to remove excess sugar through urine. Beyond glycemic control, they offer significant cardiovascular and renal protection, reducing hospitalizations for heart failure and slowing chronic kidney disease progression[8].

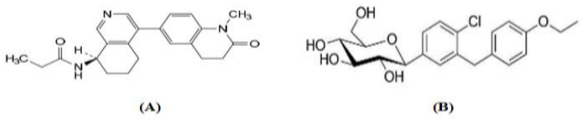

Figure 1: Chemical Structure: (A) Baxdrostat and (B) Dapagliflozin

The potential therapeutic synergy of combining these agents may offer improved clinical outcomes in CKD patients with concomitant hypertension and metabolic disorders. Clinical investigations have demonstrated that the Baxdrostat and Dapagliflozin combination is safe, well tolerated, and more effective than Dapagliflozin monotherapy in slowing CKD progression, as assessed by changes in estimated glomerular filtration rate (eGFR) over time [9, 10]. Additionally, the combination showed superior reductions in urine albumin-creatinine ratio (UACR) and systolic blood pressure (SBP), indicating enhanced renoprotective and antihypertensive effects [10]. Recognizing its clinical potential, M/s AstraZeneca has received approval from the Central Drugs Standard Control Organization (CDSCO), India [11], conducted a Phase III clinical trial evaluating Baxdrostat in combination with Dapagliflozin tablets (CT/22/24; Online Submission No. 41857). The Phase III study (Protocol No. D6972C00003, Version 2.0 dated 12 January 2024) was reviewed by the Subject Expert Committee (SEC–Renal) in its 2nd/24 meeting held on 14 March 2024 at CDSCO Headquarters, New Delhi, and permission was granted to proceed with the trial [9, 10].

Despite the growing clinical interest in this novel combination, there is currently no any validated analytical method reported yet. In this context, the development of accurate, precise, reliable and sensitive analytical methods for the simultaneous estimation of Baxdrostat and Dapagliflozin is essential for quality control, formulation development, and future pharmacokinetic investigations. Therefore, the present study focuses on the development and validation of novel analytical approaches for the simultaneous estimation of Baxdrostat and Dapagliflozin in synthetic mixture in accordance with regulatory ICH Q2 [R2] guideline [12] with validation parameters [13]. A comprehensive literature survey indicated that Baxdrostat has been analyzed using an analytical quality-by-design (AQbD) based RP-HPLC method [14], while Dapagliflozin has been extensively quantified by UV spectrophotometry [15, 16], RP-HPLC for bulk and tablet dosage forms [17], and stability-indicating HPLC methods for API and formulations[18]. Simultaneous estimation methods for Dapagliflozin with Saxagliptin have also been reported [19], along with HPTLC methods [20,21] and LC-MS/MS techniques for plasma and biological matrices [22, 23]. However, no validated analytical method has yet been reported for the simultaneous estimation of Baxdrostat and Dapagliflozin in synthetic mixture. This clear gap in the literature underscores the necessity for the development of a novel, reliable, and validated analytical approach for this emerging therapeutic combination.

2. MATERIALS:

All chemicals and reagents used in the present study were HPLC grade and analytical grade to ensure accuracy and reproducibility of the results. Acetonitrile, methanol, and water (HPLC grade) were procured from Finar Chemicals Pvt. Ltd., India, used for the preparation of mobile phases, diluents, and standard solutions. Ortho phosphoric acid of analytical reagent (AR) grade, obtained from Astron Chemical India, was employed for pH adjustment of the phosphate buffer. Baxdrostat, used as the reference standard, was kindly supplied by Torrent Research Centre Pvt. Ltd., Ahmedabad, while Dapagliflozin was procured from Stallion Pharmaceuticals Ltd., Ahmedabad.

2.1 Instruments & Software

The spectrophotometric measurements were

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

performed using a UV-Visible spectrophotometer (Shimadzu-1900, UV Probe 2.7 version software) with a spectral bandwidth of 1 nm was employed for all spectroscopic measurements, using a pair of 1.0 cm matched quartz cells over the range of 200-400 nm. For chromatographic information acquisition and analysis, High-Performance Liquid Chromatography system Systronic RP-HPLC (LC-20-AD) (SPD-20 A) with UV Detector was utilized together. The pH of the buffer solution was observed utilizing the Chemi Line pH meter. The Scale-Tec analytical balance was utilized to weigh the samples. The HPLC mobile phase was subjected to sonication using an Sonicator-Digital Pro^{+}, PS-10A, (Broleo).

2.2 Analytical conditions

In accordance with ICH Q2 (R2) requirements [12], the analytical conditions for a simultaneous technique for the measurement of Baxdrostat and Dapagliflozin in UV and HPLC were optimized and validated. For UV Spectroscopy Methanol was used as a Solvent. Detection wavelength (λmax) of Baxdrostat and Dapagliflozin were 232 nm and 224 nm, respectively. The first-order derivative UV spectra were derived from the zero-order spectra using methanol as the solvent. Quantitative analysis was performed at the zero-crossing point (ZCP) of Baxdrostat at 232 nm for the estimation of Dapagliflozin, and at the ZCP of Dapagliflozin at 255 nm for the estimation of Baxdrostat. For RP-HPLC, Kromstar C_{18} (250 mm × 4.6 mm, 5 µm) was used in the procedure. that the mobile phase 0.1% formic acid in Acetonitrile: Phosphate buffer (pH 3.4 adjusted with Ortho phosphoric acid) (20:80 %v /v) shows good peak shape and resolution. 232 nm wavelength was selected for RP-HPLC, with 1 mL/min flow rate.

2.3 Preparation of Solutions

2.3.1 Preparation of Stock Solution: Accurately weighed 10 mg of Baxdrostat and 10 mg of Dapagliflozin were individually transferred into separate 100 mL volumetric flasks and dissolved in methanol. The solutions were sonicated to ensure complete dissolution, and the volume was made up to the mark with methanol to obtain standard stock solutions having a concentration of 100 µg/mL of Baxdrostat and 100 µg/mL of Dapagliflozin, respectively.

2.3.2 Preparation standard solution: Pipetted out 0.1 ml solution of Baxdrostat (100 µg/ml) and 2 ml standard stock solution of Dapagliflozin (100 µg/ml) into different 10 ml volumetric flask and diluted up to mark with Methanol to get the 1 µg/ml of Baxdrostat and 20 µg/ml of Dapagliflozin.

2.3.3 Preparation of standard working solution: The concentration ranges of 0.5-2.5 µg/mL of Baxdrostat and 10-50 µg/mL of Dapagliflozin formed, from each stock solution, Baxdrostat (0.05, 0.10, 0.15, 0.20, and 0.25 ml) and Dapagliflozin (1, 2, 3, 4 and 5 ml) were pipetted out in ten different 10 ml volumetric flasks and made up to mark with Methanol to obtained 0.5, 1, 1.5, 2, and 2.5 µg/ml of Baxdrostat and 10, 20, 30, 40 and 50 µg/ml for Dapagliflozin, respectively. Under the optimized spectrophotometric conditions, the samples were analyzed using a 1 cm quartz cuvette in the UV spectrophotometer. Similarly, the optimized chromatographic conditions, 20 µL of each standard working solution were injected into RP-HPLC system by Hamilton syringe and analyzed.

3. METHODOLOGY

3.1 Method development

3.1.1 Method I: UV-spectrophotometric method: A first-order derivative spectrophotometric technique was employed for the simultaneous quantification of Baxdrostat and Dapagliflozin in a synthetic mixture. Separate working standard solutions of each drug were scanned within the 200–400 nm wavelength range to generate their derivative spectra, enabling the determination of appropriate zero-crossing wavelengths for accurate quantitative analysis. Baxdrostat and Dapagliflozin standard stock solutions were prepared in Methanol at concentrations of 100 µg/mL and 100 µg/mL, respectively. Appropriate volume, 0.1 mL of Baxdrostat and 2 mL Dapagliflozin from standard stock solution were transferred to two separate 10 mL volumetric flasks and the volume was adjusted to mark with methanol to get concentration 1 and 20 µg/mL, respectively. The solutions were scanned separately in the UV-region i.e., 400-200 nm. The zero-order UV absorption spectra of Baxdrostat and Dapagliflozin in Methanol shown in Figure 2 (A). The zero-order spectrum was processed to obtain first-derivative spectrum. The two first derivative spectra were overlaid which showed that Baxdrostat showed zero crossing at 232 nm, while Dapagliflozin showed zero crossing at 255 nm which showed in Figure 2 (B). The determinations were made at 255 nm for Baxdrostat (ZCP of Dapagliflozin) and 232 nm for Dapagliflozin (ZCP of Baxdrostat). The zero order and first order overlay UV spectra of Baxdrostat and Dapagliflozin showed in Figure 2 (A) and (B), respectively.

3.1.2 Method II: Reverse Phase High Performance Liquid Chromatography Method: The isocratic analysis was carried out using Reverse phase chromatographic technique because of its recommended use for ionic and moderate to non-polar compounds using a mobile phase comprised of 0.1% formic acid in Acetonitrile: Phosphate buffer (pH 3.4 adjusted with Ortho phosphoric acid) (20:80 %v /v) at a flow rate of 1 mL/min found better separation of both the drug peaks. Prior to usage, the solvents were filtered through a 0.45 µ filter and sonicated for 30 min. The stationary phase was a Kromstar C_{18} (250

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

mm × 4.6 mm, 5 µm), and the eluent was observed by a U.V Detector at 232 nm (figure 2A).

3.2 Method Validation: The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), *ICH Q2(R2): Validation of Analytical Procedures* [12] established standards for the validation of the analytical procedures utilized in this investigation.

3.2.1 Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Its results showed in figure 5.

3.2.2 Linearity and Range (n=6): The linearity of Baxdrostat and Dapagliflozin was found to be in the range of 0.5-2.5 µg/mL and 10-50 µg/mL, respectively. For the UV spectrophotometric method, calibration curves were constructed by plotting absorbance versus concentration (µg/mL). In the HPLC method, calibration curves were obtained by plotting peak area against the corresponding concentrations of Baxdrostat and Dapagliflozin. The linear regression equations were subsequently derived, and linearity for both drugs was evaluated in terms of slope, intercept, and correlation coefficient (R^2).

3.2.3 Precision: The Intraday and Interday precisions also referred to as repeatability and intermediate accuracy, respectively were used to assess the precision of Methods I and II. The experiment was conducted on the same day and for the next three days for both Intraday and Interday precision, analysing freshly made solutions at concentrations of 0.5, 1 and 1.5 µg/mL of Baxdrostat and 10, 20, and 30 µg/mL of NAC. To assess intermediate precision, the mean absorbance (UV) and peak area (HPLC) were recorded for each set of experiments. For repeatability, 1 µg/mL of Baxdrostat and 20 µg/mL of Dapagliflozin were used. The results were represented as a percentage Relative Standard Deviation (RSD), with a value of less than two considered acceptable. This meticulous approach ensures a comprehensive evaluation of the precision of the analytical methods, providing confidence in the reliability and consistency of the results obtained for the concentrations of Baxdrostat & Dapagliflozin in the tested solutions.

3.2.4 Limit of Detection (LOD): Limit of detection can be calculated using following equation as per ICH Q2 (R2) guideline.

$$\mathrm{LOD} = 3.3 * \frac{\sigma}{S}$$

where, **σ** = Standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

3.2.5 Limit of Quantification (LOQ): Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

$$\mathrm{LOQ} = 10 * \frac{\sigma}{S}$$

3.2.6 Accuracy (Recovery study) (n=3) **:** The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH Q2 (R2) guideline at three different concentration levels 50 %, 100 %, 150 % and the values were measured for Baxdrostat (1 µg/mL) and Dapagliflozin (20 µg/mL). This performance was done in triplicate.

3.2.7 Assay as analysis of Synthetic Mixture: The synthetic mixture of Baxdrostat and Dapagliflozin was prepared in the ratio of 1: 20. Common excipients such as Lactose (18 mg), Starch (9.5 mg), Magnesium Stearate (1 mg), and MCC [Micro Crystalline Cellulose] (6 mg), Croscarmellose Sodium (5 mg) were added in the motor pestle along with the drugs. Accurately weighed equivalently weight of Baxdrostat (0.5 mg) and Dapagliflozin (10 mg). All the components were transferred into a mortar and blended thoroughly using a pestle to obtain a homogeneous synthetic mixture and transferred in 100 ml volumetric flask and allow to sonicate and made up to mark with Methanol. This solution was filtered through Whatmann filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 5 µg/ml of Baxdrostat and 100 µg/ml of Dapagliflozin.

3.2.7.1 Preparation of sample solution: Accurately 2 ml of the above [mixture solution of Baxdrostat (5 µg/mL) and Dapagliflozin (100 µg/mL)] was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Baxdrostat was 1 µg/ml and Dapagliflozin 20 µg/ml.

3.2.8 Robustness: The robustness of analytical methods becomes evaluated to decide their ability to face up to minor variations in approach situations. For the HPLC technique, samples have been subjected to evaluation below changed situations, which include adjustments inside the flow rate (± 0.1 mL/min), detection wavelength (± 2 nm), and natural content material (± 2 %) inside the mobile segment. The resulting results on machine suitability parameters have been intently monitored. In the times of Methods I and II, distinct analysts conducted sample analyses to evaluate the robustness of the strategies.

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

3.2.9 System Suitability Tests: A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test included the resolution, column efficiency (theoretical plates) and tailing factor. Its results showed in Table 1.

3.3 Statistical Comparison of Methods: Statistical analysis was performed to identify significant differences among the developed analytical methods. A statistical analysis was conducted using the student's t-test [24-25] to compare the results of accuracy and assay for proposed UV spectrophotometric and HPLC methods. A statistical test (Student's *t*-test) [24-25] was applied to evaluate the significance of difference between the two methods. The calculated *t*-value was compared with the theoretical *t*-value at a 95% confidence level. The student's *t*-test was calculated using the following formula:

$$t = \frac{|\bar{X}_1 - \bar{X}_2|}{Sp\sqrt{(\frac{1}{n_1} + \frac{1}{n_2})}}$$

where, $\bar{X}_1$ and $\bar{X}_2$ were Mean value obtained from UV and RP-HPLC methods as two groups, n1 and n2 were number of observations for both methods, respectively and Sp was pooled standard deviation. The pooled standard deviation was calculated using the formula:

$$Sp = \sqrt{\frac{(n_1 - 1)(S_1)^2 + (n_2 - 1)(S_2)^2}{n_1 + n_2 - 2}}$$

Where, S_1 and S_2 were standard deviation of the proposed methods as two groups.

4. RESULTS:

4.1 Method I: UV Method: In pharmaceutical analysis, the simultaneous estimation of multiple components using UV spectroscopy is a widely utilized method. Various techniques, including the Simultaneous Equation, Derivative Spectrophotometric approach and the absorbance ratio method, are employed for this purpose. The simultaneous estimation using UV visible spectroscopy offers several advantages, including ease of use, cost-effectiveness, and minimal time and labor requirements. These attributes made UV visible spectroscopic methods particularly valuable in pharmaceutical research and quality control, allowing for efficient and economical simultaneous determination of multiple components in a given sample.

4.1.1 Selection of wavelength for Baxdrostat and Dapagliflozin: The remarkable absorbance of Baxdrostat exhibited an absorption maximum at 255 nm (**Figure 2-A**), while Dapagliflozin showed an absorption maximum at 232 nm (**Figure 2-B**). The zero-order and First Order UV absorption spectra of Baxdrostat (1 µg/mL) and Dapagliflozin (20 µg/mL) in Methanol was showed in Figure 2 (A) and 2 (B), respectively.

4.1.2 First order derivative UV Method Development: The Baxdrostat and Dapagliflozin overlapping absorption throughout the 200 - 400 nm range is shown by these spectra, which makes it more difficult to quantify the pharmaceuticals using traditional UV spectrophotometry without accounting for the overlap. The sum of the absorbances of the two compounds may be used to calculate the overall absorbance of a solution containing a combination of both at a certain wavelength. In situations where the levels of the two medicinal drugs overlap, the method entails figuring out the quantity of each drug using their zero-order spectra. The resulting absorbance spectra were derived to eliminate the interference of absorbing species. The first derivative corresponding to each absorption spectrum of each drug was recorded, using Δλ = 2 nm and scaling factor 4. The amplitude values were measured at 255 nm (λ1) (ZCP of Dapagliflozin) for Baxdrostat and 232 (λ2) (ZCP of Baxdrostat) for Dapagliflozin showed in Figure 2 (B).

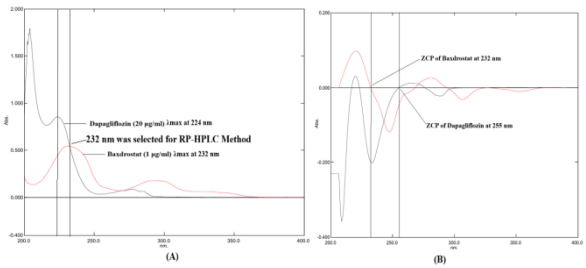

Figure 2: Overlain UV Spectra of Baxdrostat (1 µg/ml) and Dapagliflozin (20 µg/ml) in Methanol (A) (Zero Order) and (B) (First Order)

4.2 Method II: RP-HPLC Method: Pharmaceutical analysis commonly uses simultaneous estimation using RP-HPLC. It enables the use of RP-HPLC to determine the presence of many chemicals in a sample. For the simultaneous estimate of various components, including medications and their contaminants, in pharmaceutical formulations, a number of techniques have been devised and proven effective. Utilizing an appropriate column, mobile phase, and detection equipment, the simultaneous estimation technique by HPLC allows for the separation and quantification of the target substances. In pharmaceutical analysis, Reverse Phase high-performance liquid chromatography (RP-HPLC) is a great instrument for simultaneous estimation that offers confidence and specificity for the identification of chemical entities in Synthetic Mixture.

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

non-polar compounds. Reverse phase chromatography is not only simple, convenient but also performs better in terms of efficiency, stability and reproducibility. C_{18} column was selected because it is least polar compare to C_4 and C_8 columns.C_{18} column allows eluting polar compounds more quickly compare to non-polar compounds. In addition to this UV detector is used which allows easy detection of the compounds in UV transparent organic solvents. Hence, C_{18} (250×4.6 mm) column of 5µm particle packing was selected for separation of Baxdrostat and Dapagliflozin.

4.2.1 Selection of detection wavelength: The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 232 nm both drugs give good peak height and shape. So, 232 nm was selected for simultaneous estimation of Baxdrostat and Dapagliflozin in synthetic mixture.

Overlay UV spectra of Baxdrostat (1 µg/ml) and Dapagliflozin (20 µg/ml) in Methanol has been shown in Figure 4.

4.2.2 RP-HPLC Method Development: Liquid chromatography coupled with UV detection was used to develop a way for simultaneously measuring Baxdrostat and Dapagliflozin. Achieving acceptable peak symmetry and theoretical plates within a realistic time period was the aim. The chromatographic conditions were optimized by experimenting with various stationary and mobile phases. The mobile phase 0.1% formic acid in Acetonitrile: Phosphate buffer (pH 3.4 adjusted with Ortho Phosphoric acid) (20:80 %v/v) was selected because it was found to ideally resolve the peaks with retention time 2.8 min and 6 min for Baxdrostat and Dapagliflozin, respectively showed in figure 3. Kromstar C_{18} (250×4.6 mm, 5 µm) column was used for separation of Baxdrostat and Dapagliflozin with flow rate of 1.0 ml/min.

Table 1: System suitability parameter

Parameters	Retent-ion Time	Tailing Factor	Number of Theoretical plate	Resolution
Baxdrostat	2.8 min	1.1	6819	3.8
Dapagliflozin	6 min	0.8	8437	

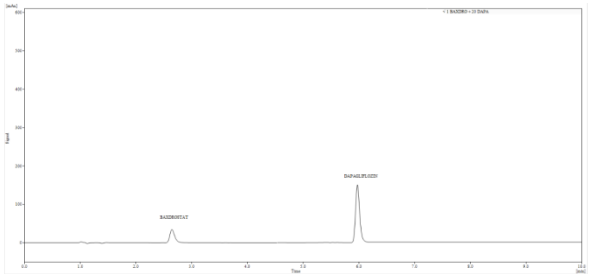

Figure 3: RP-HPLC Chromatogram of Baxdrostat (1 µg/ml) and Dapagliflozin (20 µg/ml) in in 0.1% formic acid in Acetonitrile: Phosphate buffer (pH 3.4 adjusted with Ortho Phosphoric acid) (20:80 %v/v) at 232 nm

4.3 VALIDATION OF THE PROPOSED METHODS

4.3.1 Validation Parameters of the UV Method:

4.3.1.1 Linearity and range: For Baxdrostat and Dapagliflozin, the absorbances ranged from 0.5-2.5 µg/mL at 255 nm and 10- 50 µg/mL at 232 nm showed in Figure 4 (A) and 4 (B), respectively. Linearity data of Baxdrostat and showed in Table 2.

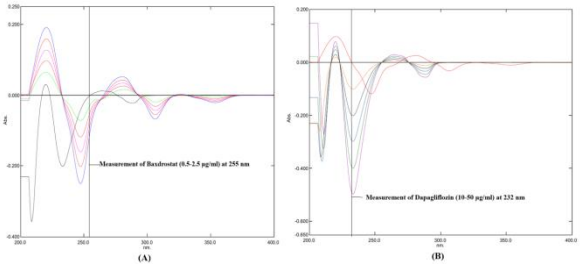

Figure 4: Overlain UV Spectra of (A) Baxdrostat (0.5-2.5 µg/ml) at 255 nm (B) Dapagliflozin (10-50 µg/ml) at 232 nm

Table 2: Linearity data of Baxdrostat and Dapagliflozin

Parameters	UV Spectrophotometry		RP-HPLC	
	Baxdrostat at 255 nm	**Dapagliflozin at 232 nm**	**Baxdrostat at 232 nm**	**Dapagliflozin at 232 nm**
Linearity Range	0.5 - 2.5 µg/mL	10 - 50 µg/mL	0.5 - 2.5 µg/mL	10 - 50 µg/mL
Regression Equation	y = -0.0278x - 0.0141	y = -0.0097x-0.0038	y = 192.81x + 22.15	y = 64.66x + 61.12
Correlation Coefficient	0.9996	0.9998	0.9995	0.9995
LOD	0.05	0.54	0.02	0.47
LOQ	0.15	1.65	0.07	1.43

4.3.1.2 Precision: In terms of precision, both Inter-day, Intraday and Repeatability measurements were conducted at three distinct concentrations 0.5, 1.0 & 2.5 µg/mL for Baxdrostat and 10, 20, & 30 µg/mL for Dapagliflozin in triplicate over three consecutive days and on the same day. The absorbance of the same solutions was measured. For repeatability, 1.0 µg/mL for Baxdrostat and 20 µg/mL for Dapagliflozin were measured. The resulting RSD values for Intraday, Inter-day precision, and Repeatability were showed in Table 3.

Table 3: Precision study of Baxdrostat and Dapagliflozin for UV Method

Intraday precision					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
BUDE	**NAC**	**Baxdrostat**	**Dapagliflozin**	**Baxdrostat**	**Dapagliflozin**
0.5	10	|-0.028| ±	|-0.104| ± 0.00162	1.40	1.56

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

		0.00039			
1.0	20	|-0.043| ± 0.00049	|-0.194| ± 0.00265	1.16	1.36
1.5	30	|-0.056| ± 0.00050	|-0.293| ± 0.00270	0.89	0.92
Interday precision					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
BUDE	**NAC**	**Baxdrostat**	**Dapagliflozin**	**Baxdrostat**	**Dapagliflozin**
0.5	10	|-0.028| ± 0.00042	|-0.103| ± 0.00164	1.40	1.58
1.0	20	|-0.043| ± 0.00051	|-0.196| ± 0.00271	1.20	1.38
1.5	30	|-0.056| ± 0.00058	|-0.293| ± 0.00282	1.04	0.96
Repeatability					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
BUDE	**NAC**	**Baxdrostat**	**Dapagliflozin**	**Baxdrostat**	**Dapagliflozin**
1.0	40	|-0.042| ± 0.00050	|-0.195| ± 0.00266	1.18	1.37

4.3.1.3 LOD and LOQ: The minimum detectable quantity of an analyte within a sample by an analytical method was determined to be 0.05 µg/mL for Baxdrostat at 255 nm and 0.54 µg/mL for Dapagliflozin at 232 nm, The quantitation limit for a specific analytical method refers to the minimum quantity of the substance in a sample that can be accurately and precisely measured which was found to be 0.15 µg/mL for Baxdrostat at 255 nm and 1.65 µg/mL for Dapagliflozin at 232 (Table 1). The low LOD and LOQ values obtained at the selected wavelengths indicated the adequate sensitivity of the proposed UV spectrophotometric method for the estimation of both drugs.

4.3.1.4 Accuracy: To decide the accuracy of the technique recuperation, change into accomplished by means of standard addition approach. To pre-analysed pattern acknowledged quantity of general Baxdrostat and Dapagliflozin spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of Baxdrostat and Dapagliflozin. Accuracy was carried out by the Recovery Studies (standard addition method). The results were stipulated in triplicate and the accuracy indicated by % recovery. For UV, The % Recovery was obtained in range of 99.26%-99.92% for Baxdrostat and 99.80%-99.94% for Dapagliflozin were showed in Table 4.

Table 4: Recovery study data for UV and RP-HPLC Method

UV Method						
Name of Drug	**% Level of recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Total Std Amt (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Mean Recovery ± SD(n=3)**
Baxdrostat	50	1	0.5	1.5	1.489	99.26±1.0100
	100	1	1	2.0	1.987	99.35±0.0800
	150	1	1.5	2.5	2.498	99.92±0.0305
Dapagliflozin	50	20	10	30	29.94	99.80±1.5033
	100	20	20	40	39.96	99.90±1.0552
	150	20	30	50	49.97	99.94±0.9556
RP-HPLC Method						
Baxdrostat	50	1	0.5	1.5	1.49	99.33±0.5465
	100	1	1.0	2.0	1.99	99.50±0.6631
	150	1	1.5	2.5	2.496	99.84±0.8243
Dapagliflozin	50	20	10	30	29.96	99.86±0.6246
	100	20	20	40	39.97	99.92±0.7564
	150	20	30	50	50.03	100.06±0.8436

4.3.1.5 Assay as Analysis of Synthetic mixture: From assay, Final concentration of Baxdrostat was 1 µg/mL and Dapagliflozin 20 µg/mL were run into UV and The Percentage assay of Baxdrostat and Dapagliflozin were found to be 99.60 % and 99.80 %, respectively. Its results showed in Table 5.

Table 5: Analysis of synthetic mixture for UV and RP-HPLC Method

UV Method				
Name of Drug	**Amount in synthetic mixture (µg/ml)**	**Mean Amount found (µg/ml)**	**% Assay ± SD (n=3)**	**%RSD**
Baxdrostat	1	0. 996	99.60 ± 1.0278	1.03
Dapagliflozin	20	19.96	99.80 ± 0.9417	0.95
RP-HPLC Method				
Baxdrostat	1	0.997	99.70±0.5682	0.57
Dapagliflozin	20	19.99	99.95±0.7624	0.76

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

4.3.2 Validation Parameters of the RP-HPLC Method:

4.3.2.1 Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. It was proved by comparing the chromatogram of mobile phase, test preparation solution to show that there was no interference of mobile phase and excipients peaks with peak of Baxdrostat and Dapagliflozin shown in figure 5.

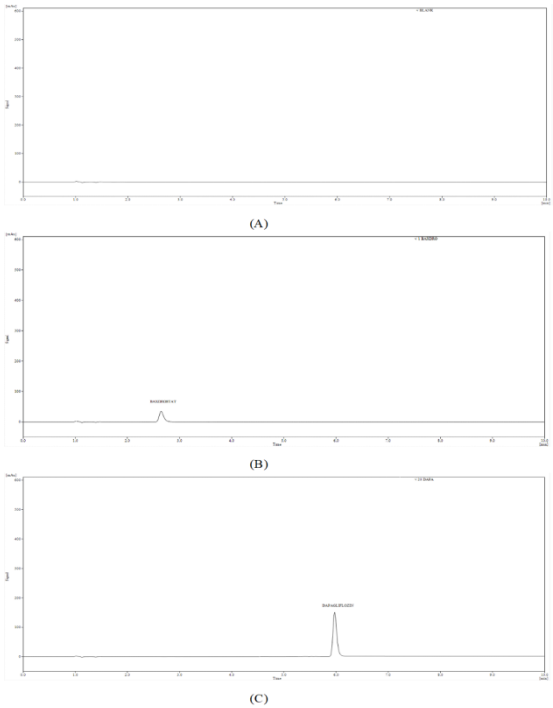

Figure 5: RP-HPLC Chromatogram (A) Blank (B) Baxdrostat (1 µg/ml) (C) Dapagliflozin (20 µg/ml) in 0.1% formic acid in Acetonitrile: Phosphate buffer (pH 3.4 adjusted with Ortho Phosphoric acid) (20:80 %v/v) at 232 nm

***4.3.2.2 Linearity*:** The RP-HPLC chromatogram of Dapagliflozin (10-50 µg/mL) and Baxdrostat (0.5-2.5 µg/mL) at 232 nm showed in figure 6. The Peak Area was found. Linearity was showed in figure 6. Calibration graphs were plotted between concentrations and peak areas. The regression equation of calibration curve was generated y = 192.81x + 22.15 for Baxdrostat and y = 64.66x + 61.12 for Dapagliflozin. The correlation coefficient (R^2) values were observed 0.9995 for both drugs.

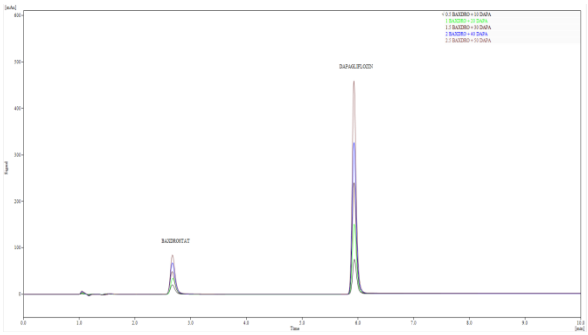

Figure 6: Overlain RP-HPLC chromatogram of Baxdrostat (0.5-2.5 µg/mL) and Dapagliflozin (10-50 µg/mL) at 232 nm

4.3.2.3 Precision: In terms of precision, both Inter-day, Intraday and Repeatability measurements were conducted at three distinct concentrations 0.5, 1.0 & 2.5 µg/mL for Baxdrostat and 10, 20, & 30 µg/mL for Dapagliflozin in triplicate over three consecutive days and on the same day. The absorbance of the same solutions was measured. For repeatability, 1.0 µg/mL for Baxdrostat and 20 µg/mL for Dapagliflozin were measured. The resulting RSD values for Intraday, Inter-day precision, and Repeatability were showed in Table 6.

Table 6: Precision study for Baxdrostat and Dapagliflozin for RP-HPLC Method

Intraday precision					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
BAX	**DAPA**	**BAX**	**DAPA**	**BAX**	**DAPA**
0.5	10	122.297±1.3257	683.735±8.9938	1.08	1.32
1.0	20	213.331±1.8088	1360.120±15.4132	0.85	1.13
1.5	30	305.571±1.9411	2032.693±19.8176	0.64	0.97
Interday precision					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
BAX	**DAPA**	**BAX**	**DAPA**	**BAX**	**DAPA**
0.5	10	121.033±1.3589	684.035±9.3738	1.12	1.37
1.0	20	216.725±1.8877	1360.053±15.4961	0.87	1.14
1.5	30	308.296±2.0446	2032.727±19.8625	0.66	0.98
Repeatability					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
BAX	**DAPA**	**BAX**	**DAPA**	**BAX**	**DAPA**
1.0	20	213.541±1.8776	1356.600±15.8966	0.88	1.17

4.3.2.4 Accuracy: The accuracy of the technique recuperation changes into accomplished by means of standard addition approach. To pre-analysed pattern acknowledged quantity of general Baxdrostat and Dapagliflozin spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of Baxdrostat and Dapagliflozin. Accuracy was carried out by the Recovery Studies

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

(standard addition method). The results were stipulated in triplicate and the accuracy indicated by % recovery. For RP-HPLC, The % Recovery was obtained in range of 99.33%-99.84% for Baxdrostat and 99.86%-100.06% for Dapagliflozin were showed in Table 4.

4.3.2.5 LOD and LOQ: The minimum detectable quantity of an analyte within a sample by an analytical method was determined to be 0.02 µg/mL for Baxdrostat at 232 nm and 0.47 µg/mL for Dapagliflozin at 232 nm, The quantitation limit for a specific analytical method refers to the minimum quantity of the substance in a sample that can be accurately and precisely measured which was found to be 0.07 µg/mL for Baxdrostat at 232 nm and 1.43 µg/mL for Dapagliflozin at 232 nm. The low LOD and LOQ values obtained at the selected wavelengths indicated the adequate sensitivity of the proposed UV spectrophotometric method for the estimation of both drugs.

4.3.2.6 Assay as Analysis of Synthetic mixture : From assay, Final concentration of Baxdrostat was 1 µg/mL and Dapagliflozin 20 µg/mL were run into UV and The Percentage assay of Baxdrostat and Dapagliflozin were found to be 99.70% and 99.95%, respectively. Its results showed in Table 5.

4.3.2.7 Robustness: Chromatographic analysis was used to analyse the effects of changes in analysts, and the results showed that there was no statistically significant difference in the% RSD of technique II. Additionally, small changes were performed to assess the robustness of the created HPLC procedures. The approaches' robustness was demonstrated by the% RSD, which remained constant despite minor variations in flow rate, run time, and detection. It was determined that the created approaches were essential as a result showed in Table 7.

Table 7: Robustness data

Condition	Variation	Baxdrostat	Dapagliflozin
		% Assay ± SD (n=3)	% Assay ± SD (n=3)
Flow rate (1 ml ± 0.2 ml/ min)	0.8 ml/min	99.42±3.5166	98.45±1.3730
	1.0 ml/min	99.65±5.2691	99.75±2.5545
	1.2 ml/min	99.76±7.4770	99.95±4.0286
Detection wavelength (232 nm ± 2 nm)	230	98.58±4.1268	99.65±2.9454
	232	99.92±4.4267	99.85±2.5055
	234	100.02±6.0256	99.99±5.3762
Mobile Phase (0.1% formic acid in Acetonitrile: PO_4 Buffer (20:80 ± 2 % v/v)	18:82	99.34±3.0784	99.47±4.1116
	20: 80	99.83±4.9421	99.65±1.0552
	22: 78	99.64±5.0143	100.05±4.1845

4.3.3 Statistical Evaluation of Analytical Methods: A statistical approach was used to differentiate between the proposed analytical approaches. There were no discernible differences among the quantities measured acquired in the subject matter evaluation through the two separate procedures based on student t-test findings. At the 5% significance level, the estimated t-value (from formula) proved less than the critical t-value (from the statistics database) and it was found that the tabulated values were greater than calculated values. So, there was no significant difference between Recovery and Assay parameters obtained through U.V. method and RP-HPLC method. Results indicated that both developed and validated analytical methods were considered accurate, precise, and statistically insignificant. The results of Compared Recovery and assay data to compare Between U.V. and RP-HPLC Methods were showed in table 8.

Table 8: Statical Comparison data of developed methods by Student t-test Analysis

Result of Compared Recovery Data		
t-test Value (Between U.V. and RP-HPLC Method)	Baxdrostat	Dapagliflozin
T calculated	0.76	0.68
T tabulated	2.12	2.12
t-test at 95% confidence interval ($p \leq 0.05$ and d.f. = 16)		
Result of Compared Assay Data		
t-test Value (Between U.V. and RP-HPLC Method)	Baxdrostat	Dapagliflozin
T calculated	0.17	0.39
T tabulated	2.78	2.78
t-test at 95% confidence interval ($p \leq 0.05$ and d.f. = 4)		

5. DISCUSSION:

The present study describes the development and validation of UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Baxdrostat and Dapagliflozin in laboratory-prepared synthetic mixtures. The validation parameters were evaluated in accordance with analytical method validation requirements. Both UV and RP-HPLC methods demonstrated excellent linearity within the selected concentration ranges. In the UV method, linearity was observed over 0.5-2.5 µg/mL for Baxdrostat at 255 nm and 10–50 µg/mL for Dapagliflozin at 232 nm, with correlation coefficients of 0.9996 and 0.9998, respectively. Similarly, the RP-HPLC method exhibited strong linearity at 232 nm with correlation coefficients of 0.9995 for both drugs. The high correlation coefficient values ($R^2 \geq 0.999$) confirm a strong linear relationship between concentration and analytical response, demonstrating that both methods are suitable for quantitative determination across the selected ranges. Precision studies (intraday, interday, and repeatability) showed %RSD values below 2% for both drugs in both

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

analytical methods. The low %RSD values indicate excellent repeatability and intermediate precision. The RP-HPLC method showed slightly lower variability compared to the UV method, reflecting its superior instrumental reproducibility. However, both techniques fall well within acceptable limits for pharmaceutical analysis. Accuracy assessed by the standard addition method at 50%, 100%, and 150% levels demonstrated percentage recoveries within 99–100% for both drugs using both methods. For the UV method, recoveries ranged from 99.26%–99.92% (Baxdrostat) and 99.80%–99.94% (Dapagliflozin). For the RP-HPLC method, recoveries ranged from 99.33%–99.84% and 99.86%–100.06%, respectively. All recovery values were within the acceptable range (98–102%), confirming the trueness and absence of interference from excipients in the synthetic mixture. For Sensitivity, the RP-HPLC method demonstrated lower LOD and LOQ values compared to the UV method, indicating comparatively higher sensitivity. However, the LOD and LOQ values obtained for the UV method were sufficiently low for routine quality control applications. These findings suggest that while RP-HPLC is more sensitive, the UV method remains adequately sensitive for quantitative estimation within the selected concentration range. For Specificity, the RP-HPLC chromatograms showed well-resolved, symmetrical peaks for both analytes with no interference from blank or excipient components. This confirms the specificity of the chromatographic method. Robustness studies for the RP-HPLC method demonstrated that small deliberate variations in flow rate, detection wavelength, and mobile phase composition did not significantly affect assay results. The %RSD values remained within acceptable limits, confirming the reliability of the developed chromatographic method. Statistical comparison using Student's t-test revealed that calculated t-values were lower than tabulated values at the 95% confidence level ($p \leq 0.05$). This indicates that there is no statistically significant difference between the UV and RP-HPLC methods in terms of recovery and assay results. Therefore, both methods are equally reliable for simultaneous estimation of Baxdrostat and Dapagliflozin. Overall, validation results confirm that both methods comply with analytical validation criteria and are suitable for routine pharmaceutical analysis.

6. CONCLUSION:

The developed UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Baxdrostat and Dapagliflozin were successfully validated in accordance with analytical validation ICH Q2 (R2) guideline. Both methods demonstrated excellent linearity, precision, accuracy, sensitivity, and specificity within the selected concentration ranges. The %RSD values were below 2%, and recovery results were within acceptable limits (98–102%), confirming the reliability and reproducibility of the methods. Statistical analysis further confirmed that there was no significant difference between the two methods. The RP-HPLC method exhibited higher sensitivity and specificity, making it more suitable for detailed quantitative and stability studies. However, the UV spectrophotometric method offers advantages of simplicity, rapid analysis, lower operational cost, and minimal solvent consumption, making it ideal for routine quality control laboratories. Therefore, both validated methods can be effectively applied for the simultaneous determination of Baxdrostat and Dapagliflozin in synthetic mixtures.

ACKNOWLEDGEMENT:

The authors are grateful to Smt. N. M. Padalia Pharmacy College, Ahmedabad, for encouragement and for providing the necessary facilities to carry out this research work.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

REFERENCES:

1. Bikbov B, Purcell CA, Levey AS, Smith M, Abdoli A, Abebe M, Adebayo OM, Afarideh M, Agarwal SK, Agudelo-Botero M, et al. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2020;395(10225):709-733.
2. World Health Organization. Global health estimates 2019: deaths by cause, age, sex, by country and by region, 2000–2019. Geneva: World Health Organization; 2020.
3. India State-Level Disease Burden Initiative Collaborators. The burden of chronic kidney disease in India: estimates from the Global Burden of Disease Study 2019. Lancet Glob Health. 2020;8(10): e1288–e1299.
4. Anjana RM, Deepa M, Pradeepa R, Mahanta J, Narain K, Das HK. Prevalence of chronic kidney disease in India: results from the ICMR-INDIAB study. Indian Journal of Medical Research. 2023;157(3):239-251.
5. Hussain MS, Maqbool M, Shaikh NK, Agrawal M, Sultana A. Therapeutic landscape of diabetic nephropathy: insights from long noncoding RNAs. Clinical Diabetology. 2024;13(5):307-318. doi:10.5603/cd.99266. Available from: https://journals.viamedica.pl/clinical_diabetology/article/view/99266.
6. Dogra S, Shah S, Gitzel L, Pusukur B, Sood A, Vyas AV, Gupta R. Baxdrostat: A novel aldosterone synthase inhibitor for treatment-resistant hypertension. *Current Problems in Cardiology*, 2023, 48(11): 101918.
7. Dhillon S. Dapagliflozin: A review in type 2 diabetes. *Drugs*, 2019, 79(10): 1135-1146. doi: https://doi.org/10.1007/s40265-019-01148-3.
8. Shaikh NK, Bhangale JO. Analytical methods for detecting and quantifying Vildagliptin and Remogliflozin in bulk and combined dosage form and their potentiality, Journal of Molecular Science, 2025, 35(3): 1100-1107. https://doi.org/10.004687/1000-9035.2025.147.
9. ClinicalTrials.gov. A phase III study to investigate the efficacy and safety of Baxdrostat in combination with Dapagliflozin on CKD progression in participants with CKD and high blood pressure. October 2024. ClinicalTrials.gov Identifier: NCT06268873. Available from: https://clinicaltrials.gov/study/NCT06268873.
10. AstraZeneca Clinical Trials. A Phase III, randomized, double-blind study to evaluate the efficacy and safety of Baxdrostat in

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1739-1749
DoI-10.004687/1000-9035.2025.226

combination with Dapagliflozin in participants with chronic kidney disease and high blood pressure (Protocol No. D6972C00003, Version 2.0, dated 12 January 2024). Clinical trial submission CT/22/24; Online Submission No. 41857. Available from: https://www.astrazenecaclinicaltrials.com/study/D6972C00003/.

11. Central Drugs Standard Control Organization (CDSCO). Minutes of the Subject Expert Committee (Renal) 2nd/24 meeting held on 14 March 2024 at CDSCO (HQ), New Delhi. New Delhi: CDSCO; 2024.
12. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Q2(R2): Validation of analytical procedures. Geneva: ICH; 2023: 1-38.
13. Shaikh NK, Jat R, Bhangale JO. Development and validation of stability-indicating RP-HPLC and UV method for simultaneous quantitation of Repaglinide and Sitagliptin phosphate in combination, American Journal of PharmTech Research, 2020, 10(6): 95-114. https://doi.org/10.46624/ajptr.2020.v10.i6.007.
14. Rani SJ, Mudavath SB. Method development and validation of Baxdrostat by using integrated analytical quality by design approach in RP-HPLC. Journal of Pharmaceutical Research, 2024, 13(4): 58–65. doi: https://doi.org/10.5281/zenodo.13571333.
15. Manasa S, Dhanalakshmi K, Nagarjuna RG, Kavitha B. Method development and validation of dapagliflozin API by UV spectroscopy. International Journal of Pharmaceutical Sciences Review and Research, 2014, 27(1): 270–272.
16. Tambe VB, Tajane PS, Godge RK. Analytical method development and validation of dapagliflozin by UV-spectroscopy. International Journal of Food, Agriculture and Nutrition Sciences, 2022, 11(1): 709–716.
17. Jitendra D, Sundeep K, Sajal K, Amjad K. A new RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form. International Journal of Drug Development & Research, 2017, 9(2): 48–51.
18. Mitali VV, Chirag JP, Patel MM. Development and stability indicating HPLC method for dapagliflozin in API and pharmaceutical dosage form. International Journal of Applied Pharmaceutics, 2017, 9(5): 33–41. doi: https://doi.org/10.22159/ijap.2017v9i5.19185.
19. Nima S, Vanita M, Pragnesh P. Stability indicating method development and validation for the simultaneous estimation of dapagliflozin and Saxagliptin in synthetic mixture by HPLC. International Journal of Pharmaceutical Sciences, 2018, 9(2): 112–128.
20. Karmankar SR, Tajne MR. A validated stability indicating high performance thin layer chromatographic method for the analysis of dapagliflozin in bulk drug and marketed tablet formulation. Asian Journal of Chemistry, 2019, 31(7): 1457–1460. doi: https://doi.org/10.14233/ajchem.2019.21824.
21. Suma V, Deveswaran R, Premnath S. A new high performance thin layer chromatographic method development and validation of dapagliflozin in bulk and tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 2019, 11(8): 58–63. doi: https://doi.org/10.22159/ijpps.2019v11i8.34339.
22. Aubry AF, Gu H, Magnier R, Morgan L, Xu X, Tirmenstein M, Wang B, Deng Y, Cai J, Couerbe P, Arnold M. Validated LC-MS/MS methods for the determination of dapagliflozin, a sodium-glucose co-transporter 2 inhibitor in normal and ZDF rat plasma. Bioanalysis, 2010, 2(12): 2001–2009. doi: https://doi.org/10.4155/bio.10.139.
23. Van der Aart-van der Beek AB, Wessels MA, Heerspink HJL, Touw DJ. Simple, fast and robust LC-MS/MS method for the simultaneous quantification of canagliflozin, dapagliflozin and empagliflozin in human plasma and urine. Journal of Chromatography B, 2020, 1152: 122257. doi: https://doi.org/10.1016/j.jchromb.2020.122257.
24. Shaikh NK, Jat R, Bhangale JO. Analysis of Vildagliptin and Nateglinide for simultaneous estimation using spectro-chromatographic methods, European Journal of Molecular and Clinical Medicine, 2020, 7(8): 741-755. https://ejmcm.com/article_3194.html.

Shaikh FAV, Shaikh NK, Bhangale JO. Development and validation of UV-spectrophotometric method for simultaneous estimation of Colchicine and Rosuvastatin calcium in synthetic mixture, Journal of Applied Bioanalysis, 2025, 11(12): 213-224. http://doi.org/10.53555/jab.v11si12.1676.