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Method development and validation for simultaneous estimation of dapagliflozin and bisoprolol by using stability-indicating RP-HPLC

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Abstract

A fixed-dose combination of dapagliflozin and bisoprolol is used to treat type 2 diabetes mellitus and can also help to manage blood pressure and cardiovascular risks. The research article aims to develop and validate a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of dapagliflozin propanediol monohydrate and bisoprolol fumarate. The separation is performed using a Kromasil 100 ODS column (250 mm × 4.6 mm, 5 µm), with a mobile phase consisting of 80:20 (v/v) methanol: water mixture adjusted to pH 3 with 0.1% formic acid, at a flow rate of 0.8 mL/min, and wavelength of 224 nm. The validation of the developed method was performed as per the ICH Q2 guideline. The medication underwent oxidation, photolysis, thermal degradation, acidic and alkaline hydrolysis. Separation of both the drugs takes place within 6 min. The developed method was linear from 5 to 25 µg/mL for both the drugs, and accurate as percentage recoveries ranged from 99.81 to 100.16% for dapagliflozin and 99.94 to 100.54% for bisoprolol. The results of precision were found within satisfactory limits. The results of the forced degradation investigation demonstrate that the approach is stability indicating since it can differentiate the peak of the active analyte from the degraded product. The suggested stability-indicating RP-HPLC method is robust, specific, linear, accurate, and precise. This approach can therefore be used effectively for stability investigations and routine analysis.

Keywords Dapagliflozin, Bisoprolol, RP-HPLC, Method development and validation

1 Introduction

The prevalence of diabetes and hypertension is rising. Compared to similar non-diabetics, hypertension is more common among diabetics [1]. Premature microvascular and macrovascular problems are significantly more likely to occur in patients with both conditions. Controlling blood pressure (BP) aggressively lowers the risk of microvascular

and macrovascular problems. In people with diabetes, the presence of hypertension increases mortality by 7.2 and 37 times, respectively [2].

The Hypertension Optimum Trial (HOT) and the U.K. Prospective Diabetes Study (UKPDS) epidemiological study demonstrated that strict blood pressure control and early blood pressure treatment significantly reduced microvascular complications (retinopathy, nephropathy, and neuropathy) and macrovascular complications (coronary artery disease (CAD), stroke, and peripheral vascular disease) [3]. Therefore, a combination of dapagliflozin and bisoprolol is used to control blood sugar levels while reducing strain on the heart, making it suitable for people with diabetes and associated heart concerns.

Dapagliflozin propanediol monohydrate, is soluble in methanol, acetonitrile, and dimethyl sulfoxide. Sodium-glucose-co-transporter 2 (SGLT2), which is mostly found in the proximal tubule of the nephron, is specifically inhibited by dapagliflozin. Since 90% of glucose absorption in the kidneys is facilitated by SGLT2, its inhibition permits glucose excretion in the urine. Patients with type 2 diabetes mellitus can benefit from improved glycaemic management and possibly weight loss through this excretion [4].

Bisoprolol fumarate, is soluble in water, methanol, and chloroform. It blocks beta-1 adrenergic receptors in the heart. This reduces the effect of adrenaline and noradrenaline, lowering heart rate and also decreasing blood pressure [5]. The structure of dapagliflozin propanediol monohydrate and bisoprolol fumarate depicted in Fig. 1.

To determine the intrinsic stability characteristics of the active components, stress testing must be carried out [6]. The outcomes about safety, quality and efficacy were significantly impacted by manufacturing process contaminants as well as improper handling or storage that produced degradants. The peak of the active analytes can be distinguished from the deteriorated product using a reliable stability-indicating technique [7, 8]. Because of their structural similarities, dapagliflozin and bisoprolol can be difficult to estimate simultaneously using conventional approaches, posing analytical hurdles. Because of these similarities, it may be difficult to distinguish the two substances precisely during analysis due to overlapping absorption spectra in UV-Visible

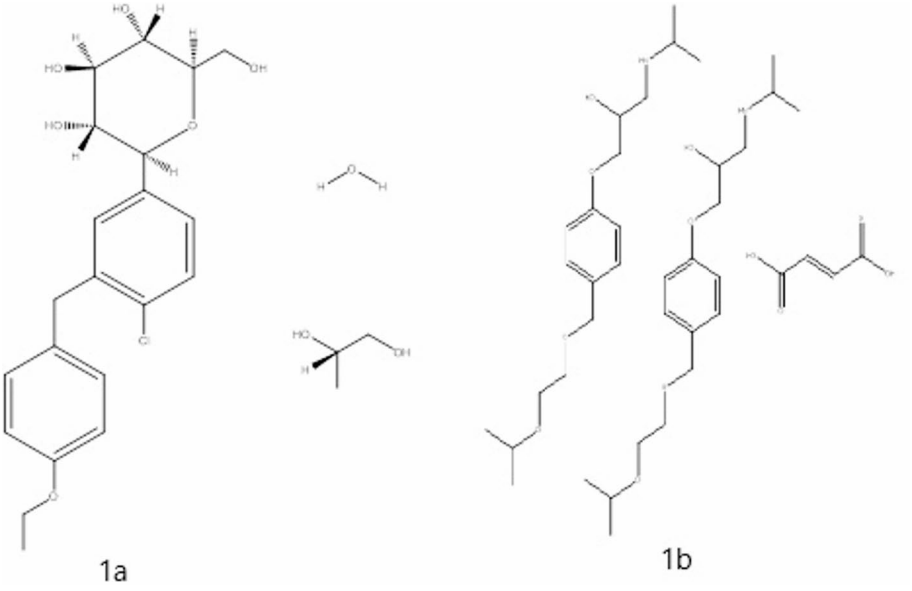

Fig. 1 Structure of **a** dapagliflozin propanediol monohydrate and **b** bisoprolol fumarate

spectrophotometry. These challenges underline the necessity for improved analytical techniques and approaches to overcome these constraints and provide an accurate and reliable estimation of both drugs in combination. The HPLC technique has been chosen for stability-indicating method development because of its many benefits, which include efficiency, accuracy, ease of use, and the ability to provide the best separation of analytes in the presence of excipients and degradants [9]. Several HPLC methods [10–20], UV-Visible spectroscopic methods [21, 22], LC-MS/MS methods [23, 24], HPTLC [25–30], stability indicating LC [31, 32] were reported in literature for the analysis of the studied drugs alone or in combination with other drugs. The aim of proposed work to develop and validate stability-indicating RP-HPLC method for the simultaneous estimation of dapagliflozin and bisoprolol in bulk and synthetic mixtures.

2 Materials and methods

The reference standard drug substance of dapagliflozin propanediol monohydrate (99.98% purity) was acknowledged as a gift sample from Honour Lab Ltd., Vishakhapatnam, India and bisoprolol fumarate (99.95% purity) was procured from Supriya Life Science Ltd., Mumbai, India. The experiment was conducted using HPLC-grade water (Loba Chemical Pvt Ltd., Maharashtra India), methanol, acetonitrile (Renkem, Maharashtra India), and AR-grade formic acid (Suvidhinath Lab, Baroda, India), and potassium dihydrogen phosphate (Suvidhinath Lab, Baroda, India).

2.1 Instrumentation

The UV spectra were taken using a Shimadzu 1800 series UV spectrophotometer. Method development and validation were conducted using the Shimadzu Prominence-i- LC 2030 C plus machine with Lab Solutions software (Shimadzu technologies, Japan). The mobile phase pH was measured using the Systronics digital pH meter (model 335, India). Sartorius (CP124S, Germany) analytical balance was used for weighing substances. Mobile phase solvents were sonicated using Ultrasonicator (Frontline Electronic and Machinery Pvt. Ltd., Ahmedabad, India).

2.2 Chromatographic condition

The separation was carried out using an isocratic mobile phase made up of methanol: water, pH 3 adjusted using 0.1% formic acid in an 80:20, v/v ratio, a flow rate of 0.8 mL/min, ODS column (250 mm × 4.6 mm, 5 µm), and a detection wavelength of 224 nm. After passing through a 0.45 µm membrane filter, the mobile phase solvent was degassed in an ultrasonic bath.

2.3 Preparation of standard solution

An accurately weighed 5 mg of dapagliflozin propanediol monohydrate, and 5 mg bisoprolol fumarate were transferred into a 50 mL volumetric flask separately. The drugs were dissolved in enough methanol, and the remaining volume was filled with methanol to reach a concentration of 100 µg/mL of dapagliflozin propanediol monohydrate and 100 µg/mL of bisoprolol fumarate.

2.4 Preparation of linearity range of dapagliflozin propanediol monohydrate (5–25 µg/mL) and bisoprolol fumarate (5–25 µg/mL)

To obtain a concentration in the range of 5–25 µg/mL for dapagliflozin propanediol monohydrate (DAPA) and 5–25 µg/mL for bisoprolol fumarate (BISO), a precisely measured volume of 0.5–2.5 mL of the standard stock solution of both medications was placed into a succession of 10 mL volumetric flasks. The volume was then adjusted with methanol. Peak area was measured after the resultant solutions were injected into a chromatographic apparatus. Plotting the calibration curve for peak area versus concentration was done.

2.5 Estimation of dapagliflozin propanediol monohydrate and bisoprolol fumarate in a synthetic mixture

A synthetic mixture was prepared by taking a standard drug in a ratio of 10 mg of dapagliflozin propanediol monohydrate, 10 mg of bisoprolol fumarate, and other excipients such as 40 mg of lactose, 1.5 mg of talc, 3 mg of magnesium stearate, 4.5 mg of polyvinylpyrrolidone, and 80 mg of microcrystalline cellulose, and transferring it into a 50 ml volumetric flask and adding enough methanol to dissolve the drugs, and bring the volume up to mark with methanol. After 10 min of ultrasonication, the test solution was filtered using 0.45 µm Whatman filter paper. Take 0.5 mL of the above test solution into a 10 mL volumetric flask, and dilute up to the mark with methanol. The test solution was injected into the HPLC system, and the peak area from the chromatogram and the percentage of DAPA and BISO was calculated.

2.6 Method validation

The Analytical method was validated according to the International Council on Harmonization (ICH) guideline Q2 (R2).

2.6.1 Linearity

The five different concentrations (5–25 µg/mL) of DAPA and BISO were injected into the HPLC system to plot a linearity curve and determine the correlation coefficient.

2.6.2 Precision

A precision study was executed at the intraday and interday levels by analysing three different concentrations and three repetitions of each concentration covering the specified range for the procedure, and determining the standard deviation and relative standard deviation.

2.6.3 Accuracy

Different concentration levels (80%, 100%, and 120%) of DAPA and BISO were constructed by spiking a standard solution into a test solution, and the results should be shown as % recovery.

2.6.4 Limit of detection and limit of quantification

The average slope (S) and the standard deviation (SD) of the intercept were used to measure the LOD and LOQ by using the following equations. $LOD = 3.3\ \delta/S$, $LOQ = 10\ \delta/S$, where S is the slope of the calibration curve and δ is the standard deviation.

2.6.5 Robustness

Robustness assesses the process's consistency across varying conditions, and its results fall within an acceptable range of accuracy, and precision. Robustness was established by changing the pH (± 0.2 pH units) and composition of the mobile phase (± 5% organic), flow rate (± 0.1 mL/min), and detection wavelength (± 2 nm). The developed approach would only be deemed appropriate if every variable matched the system's appropriateness requirements.

2.6.6 System suitability

To make sure the analytical method is appropriate for the suggested analytical procedure, a system suitability analysis was conducted. A variety of system appropriateness factors were examined, including tailing factor, column theoretical plates, retention time, and resolution.

2.6.7 Specificity

Specificity demonstrates that a method can correctly detect and quantify the drug even when other substances may be present. It helps separate the analyte from other compounds and makes sure that outside influences don't affect the detection. Specificity was confirmed by comparing the chromatograms of test, standard, and blank to check interferences.

2.7 Forced degradation studies

To assess the stability indicator property of the proposed HPLC method, both medications were subjected to severe degradation conditions, including heat, base, oxidation, acid, and photolytic degradation. In all degradation studies, the deterioration product and the percentage area of DAPA and BISO were taken into account and used for computation.

2.7.1 Acid degradation

1 mL of standard solution of DAPA and BISO was transferred into a 10 ml of volumetric flask. The solution was treated with 1 ml of a 0.01 N HCl, mixed well, and kept aside for 2 h at RT (25 °C). The solution was neutralised with 0.01 N NaOH solution and diluted up to the mark with methanol. After passing through a 0.20 µm PTFE syringe filter, the solution was subjected to further analysis using the suggested methodology.

2.7.2 Base degradation

1 mL of standard solution of DAPA and BISO was transferred into a 10 mL of volumetric flask. The solution was treated with 1 mL of a 0.01 N NaOH, mixed well, and kept aside for 2 h at RT (25 °C). The solution was neutralised with 0.01 N HCl solution and diluted up to the mark with methanol. After passing through a 0.20 µm PTFE syringe filter, the solution was subjected to further analysis using the suggested methodology.

2.7.3 Oxidative degradation

1 mL of standard solution of DAPA and BISO was transferred into a 10 mL of volumetric flask. The solution was treated with 3% H_2O_2 solution, mixed well, and kept aside for 2 h at RT (25 °C). The volume was adjusted with methanol. After passing through a

0.20 µm PTFE syringe filter, the solution was subjected to further analysis using the suggested methodology.

2.7.4 Thermal degradation

Standard drug substances of DAPA and BISO was kept at 40 °C for 30 min in a hot air oven. To obtain an appropriate concentration of both medications, 5 mg each of DAPA and BISO were dissolved in 50 mL of methanol and then further diluted. After passing through a 0.20 µm PTFE syringe filter, the solution was subjected to further analysis using the suggested methodology.

2.7.5 Photolytic degradation

Standard drug substances of DAPA and BISO was exposed to direct sunlight for 30 min. To obtain an appropriate concentration of both medications, 5 mg each of DAPA and BISO were dissolved in 50 mL of methanol and then further diluted. After passing through a 0.20 µm PTFE syringe filter, the solution was subjected to further analysis using the suggested methodology.

3 Results and discussion

3.1 Selection of detection wavelength

The standard solution of DAPA (10 µg/mL) and BISO (10 µg/mL) was scanned in a UV-visible spectrophotometer from 400–200 nm (Fig. 2). As both the drugs show good

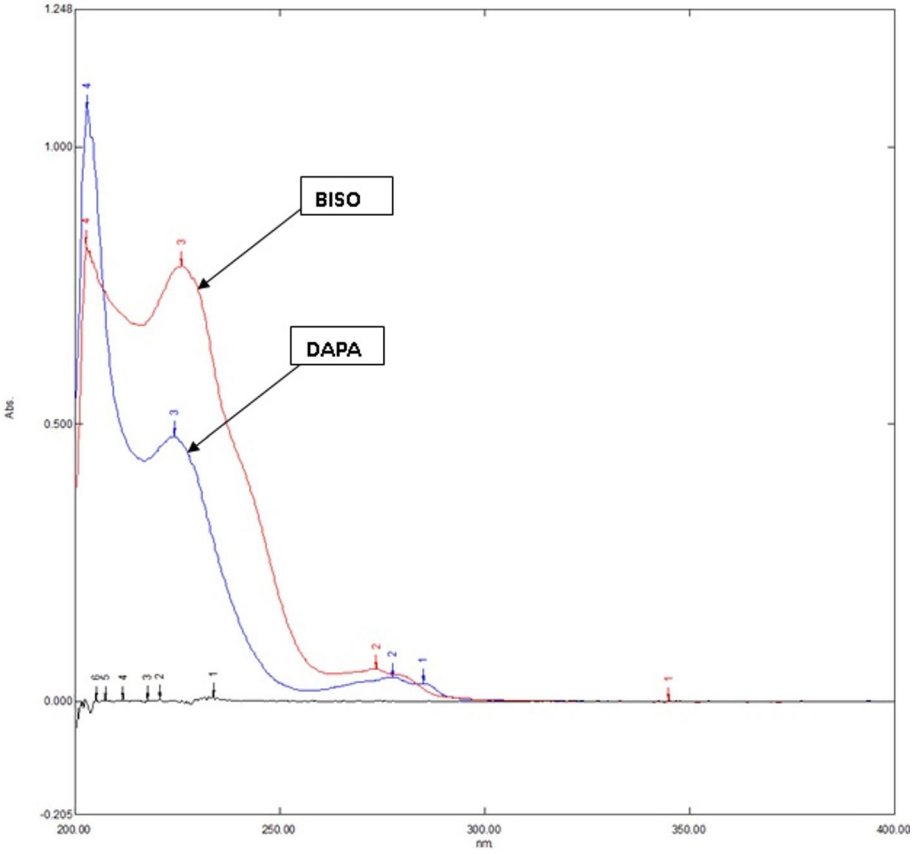

Fig. 2 Overlay UV spectra of DAPA (10 µg/mL) and BISO (10 µg/mL) in methanol

absorbance at 224 nm, which was employed for HPLC method development. The UV spectra of the standard solutions for DAPA and BISO are indicated in Fig. 2.

3.2 Optimization of mobile phase

Initially, several ratios of water to methanol, water to acetonitrile, and methanol-acetonitrile to water were studied, but poor separation was achieved. In RP-HPLC, optimization of mobile phase pH is important for developing rugged methods, because it directly impacts on retention time of analytes, broadening and symmetry of peaks. The different pH was adjusted by using formic acid to achieve proper separation. As the ratio of methanol increases a shorter retention time is observed. In the proposed RP-HPLC method, both drugs are well separated within 6 min, which leads to less solvent consumption and time. Finally, good separation with adequate resolution and a symmetrical peak of DAPA and BISO was obtained in the mobile phase of methanol with water in the ratio of 80:20, v/v, pH 3 set using 0.1% formic acid at 0.8 mL/min flow rate. The chromatogram of the standard solutions for DAPA and BISO is shown in Fig. 3. The DAPA and BISO were separated at R_t 2.10 min and 5.84 min, respectively. The peaks of DAPA and BISO were well resolved from each other with good peak shape. The results indicate that the chromatographic conditions employed were effective for the separation of the two compounds. This separation allows for accurate quantification and analysis of DAPA and BISO in subsequent experiments.

3.3 Method validation

3.3.1 Linearity

Linear responses of both drugs were evaluated between 5 and 25 µg/mL concentration. with R^2 of 0.9928, and 0.9940, respectively. The calibration curves are shown in Figs. 4 and 5. The linearity data are depicted in Table 1.

3.3.2 Precision

The within-day precision the mean % RSD values for DAPA was 0.399, and for BISO was 0.356. In the same way, the mean % RSD values for different- day was 0.392 for DAPA and 0.549 for BISO. The RSD values below 2% indicates that the developed method is

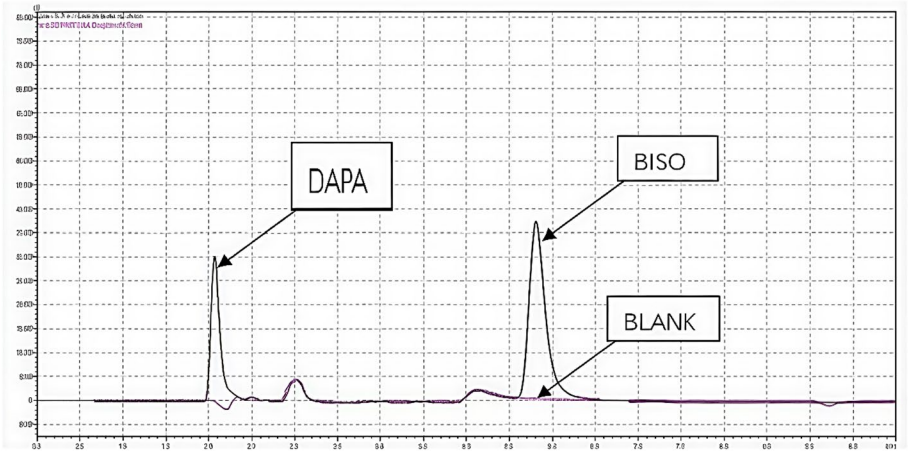

Fig. 3 Chromatogram of blank and standard of DAPA (10 µg/mL) and BISO (10 µg/mL)

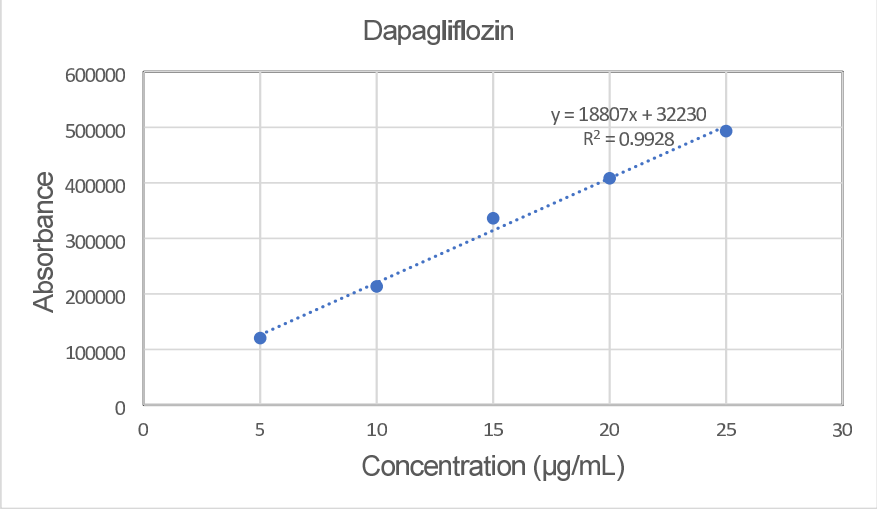

Fig. 4 Calibration curve of dapagliflozin (5–25 µg/mL)

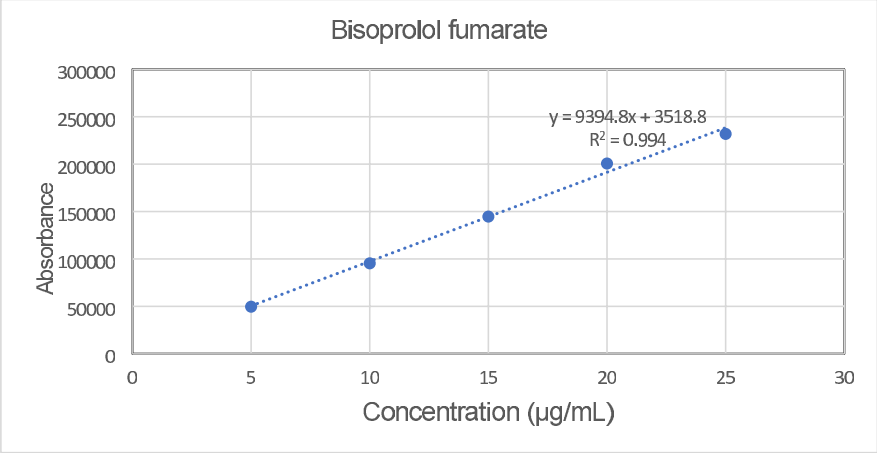

Fig. 5 Calibration curve of bisoprolol (5–25 µg/mL)

Table 1 Result of linearity

Conc (µg/mL)	Avg AUC ± SD (mAU*min) (*n* = 3)	%RSD	Conc (µg/mL)	Avg AUC ± SD (mAU*min) (*n* = 3)	%RSD
DAPA			BISO		
5	120,462 ± 308.1	0.255	5	49,635 ± 228.2	0.479
10	213,473 ± 457.3	0.214	10	95,375 ± 512.9	0.537
15	336,245 ± 573.5	0.170	15	144,680 ± 295.8	0.204
20	408,265 ± 980.8	0.240	20	200,647 ± 658.5	0.328
25	493,246 ± 934.5	0.189	25	231,870 ± 532.8	0.229

precise. Tables 2 and 3 represents the results of intraday precision and interday precision, respectively.

3.3.3 Limit of detection (LOD) and limit of quantitation (LOQ)

The high sensitivity of the method for detection and quantification was established by the outcomes. It was observed that detection and quantification values for DAPA were

Table 2 Result of intraday precision

Parameters	DAPA			BISO		
	Conc (µg/mL)					
	10	15	20	10	15	20
AUC (mAU*min)	213,321	336,110	408,999	95,013	144,206	190,321
	212,589	335,682	407,125	95,152	142,564	191,258
	211,864	333,158	405,941	95,641	143,141	191,687
Mean	212591.3	334983.3	407,355	95268.67	143303.7	191088.7
SD	728.50	1595.20	1541.92	329.86	832.99	698.57
%RSD	0.342	0.476	0.378	0.121	0.581	0.365

Table 3 Result of interday precision

Parameters	DAPA			BISO		
	Conc (µg/mL)					
	10	15	20	10	15	20
AUC (mAU*min)	218,795	335,869	415,625	96,245	143,785	194,258
	219,425	335,047	412,047	95,895	145,248	193,685
	217,548	333,951	414,823	96,467	142,338	192,958
Mean	218589.3	334955.7	414,165	96202.33	143,790	193633.7
SD	955.25	962.26	1877.56	288.38	1455	651.52
%RSD	0.437	0.287	0.453	0.299	1.011	0.336

Table 4 Average %recovery and %RSD of the drugs

Drug	%Spiked	Sample Conc (µg/mL)	standard Conc (µg/mL)	Total Conc (µg/mL)	Found Conc (µg/mL)	%recovery
DAPA	80	10	8	18	18.03	100.16
	100	10	10	20	20.01	100.05
	120	10	12	22	21.96	99.81
BISO	80	10	8	18	17.99	99.94
	100	10	10	20	20.09	100.45
	120	10	12	22	22.12	100.54

0.155 µg/mL and 0.465 µg/mL, whereas the detection and quantification values for BISO were 0.224 µg/mL and 0.680 µg/mL, respectively.

3.3.4 Accuracy

The suggested method was used to assess recovery by spiking with a standard at three concentrations levels: 80, 100 and 120%. The % recovery of DAPA and BISO was found to be 99.81 to 100.16, and 99.94 to 100.54, respectively. Accuracy data is depicted in Table 4.

3.3.5 Specificity

As there was no interfering peaks were observed in both standard and test solution, which indicates that method is specific. By comparing the chromatogram, it was observed that peak in test solution was at the same Rt value for both standard drugs: 2.10 min and 5.84 min for DAPA and BISO, respectively.

Table 5 Robustness study of the developed method

Parameter	DAPA (%RSD)	BISO (%RSD)
Flowrate: (0.8 mL/min and 1.2 mL/min)	0.255	0.368
Mobile phase composition: 75:25 (v/v) and 85:15 (v/v) methanol: water	0.254	0.144
Wavelength: (222 nm and 226 nm)	0.090	0.070
pH: (2.8 and 3.2)	0.281	0.361

Table 6 Result of system suitability

Parameter	DAPA	BISO
Peak asymmetry	1.67 ± 0.12	1.31 ± 0.02
Number of HETP	2434 ± 42	4379 ± 28
Resolution	–	2.835 ± 0.70
Retention time (min)	2.04 ± 0.05	5.74 ± 0.15

Table 7 Solution stability data

Drugs	% recovery (mean ± SD, *n* = 3) at RT		% recovery (mean ± SD, *n* = 3) at 4–8 °C	
	Day 0	Day 2	Day 0	Day 2
DAPA	99.34 ± 0.25	98.89 ± 0.47	100.15 ± 0.46	99.25 ± 0.87
BISO	101.12 ± 0.58	100.24 ± 0.57	101.65 ± 0.22	100.54 ± 0.65

3.3.6 Robustness

The percentage RSD values were used to express the robustness studies. It showed a negligible variation in outcomes, suggesting the robustness of the proposed method. Table 5 illustrates the robustness study's outcomes.

3.3.7 System suitability

System suitability investigations are used to confirm the number of HETP, peak tailing, and resolution. The resolution showed greater than 2, the tailing factor showed less than 2, and the number of theoretical plates was more than 2000, which was adequate for the analysis performed. The system suitability results are depicted in Table 6. These results indicate that the chromatographic system is performing well and can reliably separate the analytes of interest. Consequently, the method can be considered suitable for routine analysis under the specified conditions.

3.3.8 Solution stability

Solution stability data was evaluated over 24–48 h by comparing the % recovery before and after storage of QC standards at room temperature and 4–8 °C (Table 7). No significant changes in the % recovery of DAPA and BISO, nor any degradation peaks were observed. The results indicates that both drugs stable for up to 2 days at both temperatures.

3.4 Forced degradation studies

Both drugs were degraded in five different stress conditions. It was found that both medications DAPA and BISO degrade more in oxidative and thermal conditions and less in acidic hydrolysis and photolytic conditions (Table 7). Figures 6, 7, 8, 9 and 10 displays the degradation studies chromatogram.

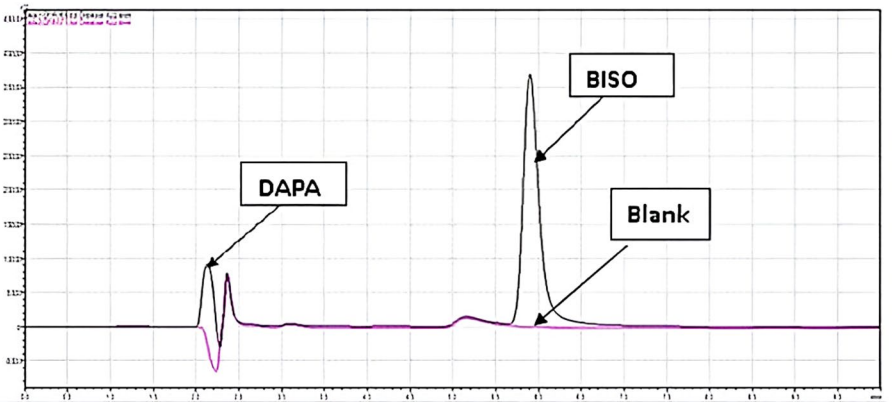

Fig. 6 Chromatogram of acid degradation

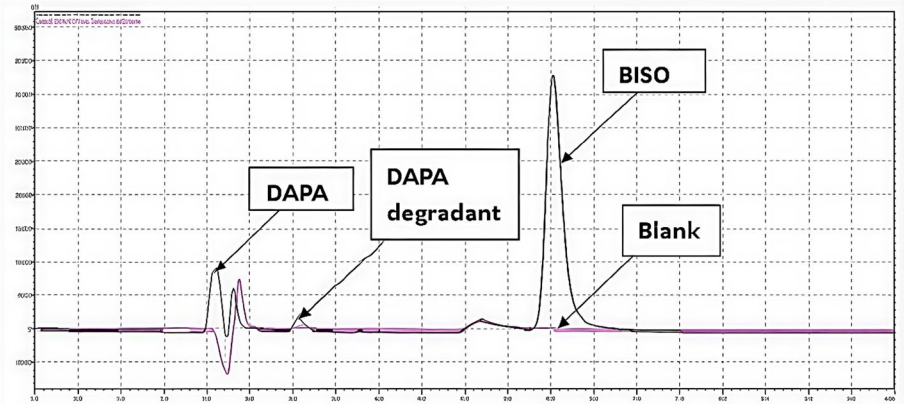

Fig. 7 Chromatogram of alkali degradation

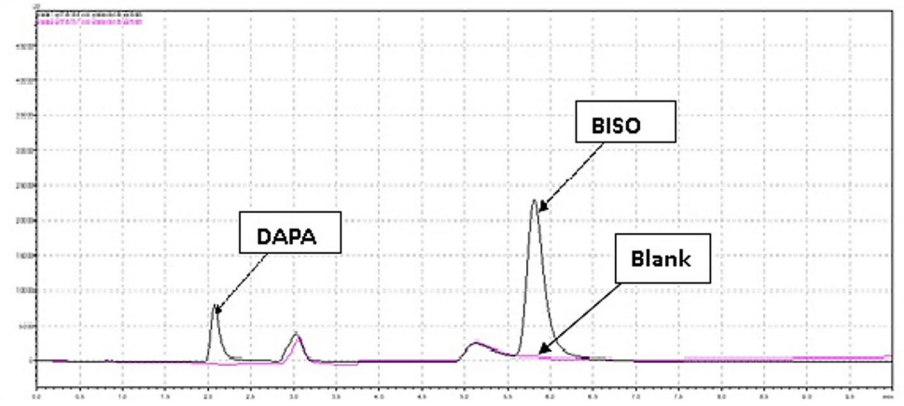

Fig. 8 Chromatogram of thermal degradation

3.5 Assay of synthetic mixture by the proposed RP-HPLC method

The synthetic mixture containing 10 mg DAPA and 10 mg BISO was analyzed using developed techniques showed that there was no interference from the excipient and the outcomes are displayed in Table 8. The results are in good agreement with contents as mentioned (Table 9).

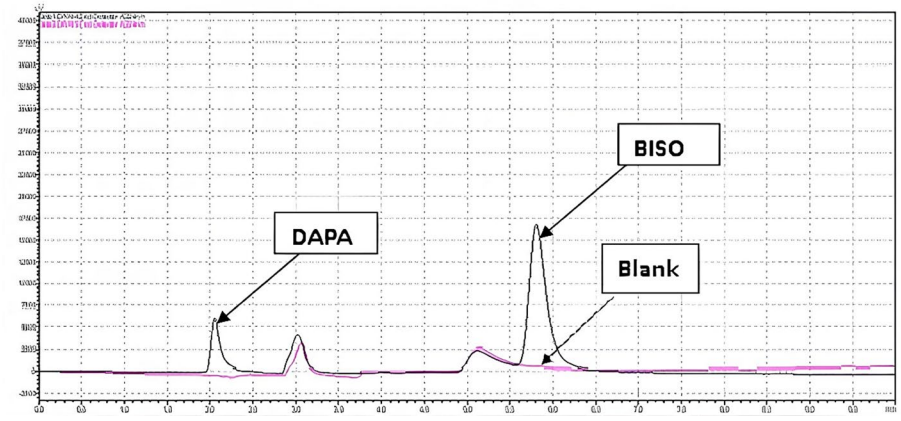

Fig. 9 Chromatogram of photolytic degradation

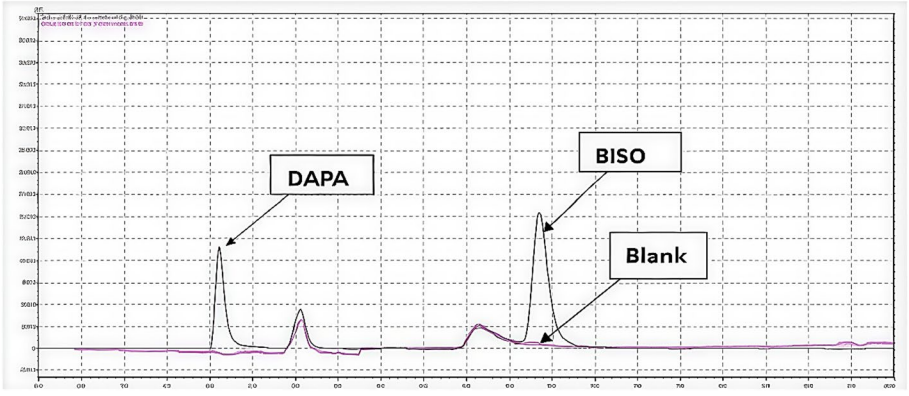

Fig. 10 Chromatogram of oxidative degradation

Table 8 Results from forced degradation study under various stress conditions

Stress type/condition	% Degradation	
	DAPA	BISO
Acid /0.01% HCL/ 2 h/ RT	3.06%	5.55%
Base/ 0.01% NaOH/ 2 h/ RT	16.85%	20.56%
Thermal/Oven/ 40 °C/ 30 min	58.35%	39.70%
Photolytic/ Sun light/ 30 min	12.72%	7.26%
Oxidative/ 3% H_2O_2/ 2 h/ RT	36.84%	34.35%

Table 9 Assay results of DAPA and BISO in synthetic mixture

Drug	Conc. taken (µg/mL)	Mean area ± SD	Conc. found (µg/mL)	% Assay ± SD
DAPA	10	216384.70 ± 5394.30	9.79	97.95 ± 0.21
BISO	10	97,298 ± 155.90	9.98	99.83 ± 0.15

3.6 Comparison of proposed HPLC method with reported HPLC method

The newly developed RP-HPLC method was compared with published methods of RP-HPLC [32] for the quantification of DAPA and BISO. The proposed RP-HPLC method utilised a simple isocratic mode of separation with a mobile phase consisting of methanol and water, in contrast to the more complex gradient elution that used a mobile phase containing buffer and acetonitrile. The results indicated that proposed method offered

Table 10 Comparison of developed RP-HPLC method with reported RP-HPLC method

Sr. No.	Drugs	Mode of separation	Retention time (min)	Linearity (µg/mL)	% recovery	Refs.
1	DAPA	Gradient	12.438	2–24	98.79–101.17	[32]
	BISO		5.712	1–12	98.92–101.20	
2	DAPA	Isocratic	2.10	5–25	99.81-100.16	Proposed method
	BISO		5.84	5–25	99.94-100.54	

simple mode (isocratic) of separation, and shorter analysis time making more efficient for routine quality control (Table 10).

4 Conclusion

The method achieved the best separation of DAPA and BISO simultaneously in isocratic mode, which eliminates the complex and tedious gradient elution process and does so without using buffer to eliminate the problem of precipitation during separation. Both the drugs are well separated in shorter time, which reduce overall analysis time and solvent consumption. This method demonstrated its capability for detecting and quantifying pharmaceuticals at lower concentrations by providing adequate figures of merit, such as excellent linearity, precision, and accuracy, and low LOD and LOQ. These results highlight the effectiveness of the method for routine analysis in pharmaceutical settings. Furthermore, the simplicity of the isocratic approach allows for easier implementation in laboratories, making it a valuable tool for researchers and quality control analysts alike.

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Author contributions
The idea was comprehended and established by RP and DS, who confirmed that the analytical method was not described. The research work was developed by DS and supervised by NT and RP. RP and DS contributed to the understanding of the results and discussion. DS and NT wrote the research paper and was supported by RP. All authors have read and accepted the final manuscript.

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Data availability
The authors state that the all data generated or analysed during this study are included in published article.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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