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Research Article

Development, Validation and Greenness Assessment of Stability Indicating RP-HPLC Method for Simultaneous Quantification of Dapagliflozin and Metoprolol Succinate in Synthetic Mixture

Sapna Rathod[1*], Ayushi Shah[1], Nisarg Patel[2]

[1]*Department of Pharmaceutical Chemistry and Quality Assurance, APMC College of Pharmaceutical Education and Research, Himatnagar, Gujarat, India.*

[2]*Department of Pharmacognosy, APMC College of Pharmaceutical Education and Research, Himatnagar, Gujarat, India.*

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ABSTRACT

A novel, rapid, accurate, precise and simple stability-indicating RP-HPLC method has been proposed and validated for the concurrent quantitation of dapagliflozin and metoprolol succinate in laboratory prepared mixture. The greenness assessment of the proposed approach was accomplished utilizing GAPI and AGREE tools. The final chromatographic run was performed utilizing Methanol: 20 mM potassium dihydrogen phosphate: (70:30) as eluent, injected at 25°C at a rate of 1.5 mL/min. Cosmosil-MS-5 C18 column (250 × 4.6 mm, 5 µm) was utilized and estimation was done using 223 nm. The APIs were exposed to stress environments (oxidation, acidic and basic hydrolysis, photolysis and thermal stimuli) and stressed samples were analyzed under the same chromatographic condition. Dapagliflozin and metoprolol succinate had retention times of 11.6 and 2.6 minutes, correspondingly. It was demonstrated to have linearity within 10 to 30 µg/mL for dapagliflozin and 50 to 150 µg/mL for metoprolol succinate. The %accuracy for dapagliflozin was in the range of 99.01 to 100.56%, while for metoprolol succinate, it was within 99.32 to 101.41% at three levels. The outcomes for the precision study were within the limits. For dapagliflozin, the LoD and LoQ were 0.49 and 0.59 µg/mL correspondingly and for metoprolol succinate, values were 1.48 and 1.79 µg/mL, correspondingly. The approach is effectively suggested for stability-indicating research as well as for routine estimation of dapagliflozin and metoprolol succinate in bulk and laboratory-prepared mixture. Furthermore, AGREE and GAPI outcomes confirmed the developed approach's eco-friendliness and greenness.

INTRODUCTION

Myocardial infarction (MI) is a common sign of coronary artery disease. In 2015, there were approximately 15.9 million myocardial infarctions globally. Of the 483 patients who received treatment between 1992 and 1996 for acute myocardial infarction (AMI), 4% showed heart failure (also known as HF) indications at the time of admission, and an additional 39% developed HF while in the hospital.[1] Cardiovascular diseases were estimated to be the cause of 17.9 million deaths globally in 2019, accounting for 32% of all deaths. Heart attacks and strokes were the cause of 85% of these fatalities. HF is the foremost predictor of death in individuals with AMI, and its effects on treatment are substantial.[2]

Dapagliflozin (DAPA; Fig. 1(A)), a sodium-glucose co-transporter - 2 (SGLT2) inhibitor[3] and metoprolol succinate (METO; Fig. 1(B)), a selective ß-1 blocker,[4] is a novel combination therapy recently approved by CDSCO for phase-3 trials.[5] Patients who had an AMI followed by HF are eligible for the combination. SGLT2 inhibitors, which block sodium-glucose co-transporter 2, have the potential

***Corresponding Author:** Dr. Sapna M. Rathod

Address: Department of Pharmaceutical Chemistry and Quality Assurance, APMC College of Pharmaceutical Education and Research, Himatnagar, Gujarat, India.

Email ✉**:** srathod456@gmail.com

Tel.: +91-9429748662

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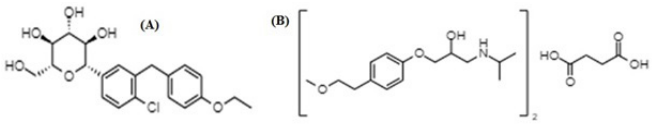

Fig. 1: Structural representation of dapagliflozin (A) and metoprolol succinate (B)

to reduce HF hospitalization, while β-blockers effectively prevent myocardial inflammation.[6]

The environmental and health risks associated with organic solvents widely employed in RP-HPLC have drawn the attention of analytical community researchers to develop an environmentally friendly RP-HPLC approach. Green chemistry aims to achieve safe solvent detoxification while reducing environmental contamination. Analytical chemists looking for greener options to substitute polluting analytical procedures, are very interested in the greening of HPLC methods. The use of dangerous chemicals must now be completely avoided, and operator- and environmentally-friendly techniques should be developed without sacrificing the accuracy of analytical methods.[7]

Studies involving forced degradation provide information on the products and pathways of degradation, contribute to analytical technique development, and enhance knowledge of the stability of the therapeutic product and drug. Obtaining adequate degraded samples is one of the key obstacles in designing a stability-indicating method (SIM). Ideally, these deteriorated samples would be real-time stability samples with all pertinent degradation products present, as well as those that are present under standard storage conditions. Stability studies are conducted to provide information on the effects of temperature, light, humidity, and other external conditions on a substance's state over time. This information helps to suggest storage conditions, reanalysis intervals, and expiration dates.[8,9]

According to the literature study, a number of HPLC analyses have been reported for the quantitation of DAPA[3,10-25] individually in dosage form or in the presence of its degradants. Similarly, some HPLC techniques were also available for the quantitative estimation of METO[4,26-31] alone in medication or when its degradants are present. Furthermore, the combination was recently approved by CDSCO for Phase III clinical trials in November 2023, there is only a single RP-HPLC method[32] reported for the concurrent quantitation of DAPA and METO in a synthetic blend. To our knowledge, no RP-HPLC SIM was reported for the concurrent quantitation of DAPA and METO in bulk and laboratory-prepared blend with greenness assessment. Hence, the purpose of the work was to establish a precise, specific and accurate SIM using RP-HPLC for the analysis of DAPA and METO in a synthetic mixture. Validation of the current approach was accomplished as per ICH Q2 (R1)[33] regulations. ICH Q1A (R2)[34] recommendations were followed in performing forced degradation testing, exposing the synthetic mixture to stress environments like oxidation, heat, acid, base, oxidation, heat, and photolysis. Furthermore, GAPI[35] and AGREE[36] tools were applied to assess the method's greenness. The current method successfully resolved the drug from degradation products formed under different stimuli.

Materials And Methods

Chemicals

DAPA (99.86% purity) and METO (99.64% purity) were procured from Torrent Pharma Ltd, Ahmedabad and Kopran Laboratories Ltd, Mumbai, correspondingly. Potassium dihydrogen phosphate (KH_2PO_4; HPLC grade) was bought from Merck Life Science Pvt. Ltd, Mumbai. Acetonitrile and Methanol (LC grade) were bought from Thermo Fisher Scientific Ltd, Ahmedabad. Sodium hydroxide (NaOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were bought from Sigma-Aldrich Chemicals Private Limited, Bangalore. Excipients utilized for the preparation of the synthetic mixture were brought from S. D. Fine Chemicals Ltd, Mumbai.

Chromatographic Conditions

Shimadzu LC 2010 CHT with PDA Detector was employed to conduct the HPLC analysis. Data was acquired using LC Solution software. Using a column Cosmosil-MS-5 C18 (250 × 4.6 mm, 5 µm) and an eluent combination of methanol: 20 mM KH_2PO_4 (70:30) pumped at 25°C at a rate of 1.5 mL/min, an isocratic separation was accomplished. A 30 µL was set as injection volume and the analytes were quantified at 223 nm.

Preparation of Solutions

Preparation of 20 mM KH_2PO_4

A 2.72 g KH_2PO_4 was accurately weighed & transferred in 1000 mL water. It was mixed well and sonicated for 10 minutes. Filtration was carried out.

Preparation of mobile phase

Methanol and 20 mM KH_2PO_4 were combined in a 70:30% v/v ratio. Filtration was done utilizing 0.45 µm membrane filter and sonicated to degas.

Standard solution preparation

Accurately 10 mg DAPA and 47.5 mg METO were weighed before being put into a 50 mL volumetric flask. The diluent utilized was methanol. This solution represents a stock solution of DAPA (200 µg/mL) and METO (1000 µg/mL), correspondingly.

Synthetic mixture preparation

The synthetic mixture equivalent to DAPA (10 mg) and METO (47.5 mg) was formulated. Conventional excipients such as HPMC, lactose, PEG 4000, cross povidone, talc and magnesium stearate were weighed appropriately and incorporated to formulate a synthetic mixture.

Sample preparation

A synthetic mixture weighing equivalent to 10 mg DAPA was taken into 50 mL of a volumetric flask (Stock solution). Dilution was done utilizing diluent with intermittent shaking (represents 200 µg/mL DAPA and 1000 µg/mL METO). Filtration was carried out. Aliquot 1-mL filtrate in 10 mL volumetric flask and diluent was utilized to achieve 20 µg/mL DAPA and 100 µg/mL METO.

Method Validation

System suitability study

A solution comprising of DAPA (20 µg/mL) and METO (100 µg/mL) in six replicates was injected into the HPLC system. 30 µL was set as the injection volume. Peak area, resolution, theoretical plates, retention time and tailing factor were noted and %relative standard deviation (RSD) was computed.

Specificity

The assessment involved injecting a placebo, standard, and sample solution to examine for excipient and analyte interference.

Linearity

The calibration curve's least square regression analysis was used to perform it. Fill 10 mL volumetric flasks with aliquots (0.5, 0.75, 1, 1.25, and 1.5 mL) of each solution (stock) of DAPA and METO separately. Methanol was used to make up the level. The final concentration encompassing 10 to 30 µg/mL DAPA and 50 to150 µg/mL METO. Graphing average peak area vs drug concentration (n = 3) yielded the calibration curve, from which the values of R^2 and y = mx + c were computed.

Precision

Precision was measured for 3 parameters, i.e., repeatability, intraday and interday precision. Using the solution of DAPA (20 µg/mL) and METO (100 µg/mL), the repeatability was examined. The solutions were analyzed six times and %RSD was computed. The intermediate precision was performed at different intervals on every day (intraday) and on three consecutive days (interday). DAPA (10, 20, 30 µg/mL) and METO (50, 100, 150 µg/mL) were analyzed thrice for intermediate precision study. The %RSD was computed for all the assessments.

Accuracy

The accuracy was performed using the placebo recovery method. The API was spiked at 80, 100, and 120% of label claims into placebo. The DAPA standard (8, 10, 12 mg) and METO standard (38, 47.5, 57 mg) were spiked into placebo. The placebo blend was transferred separately in 50 mL volumetric flask for each level. Suitable dilutions were made to ensure the final concentration within linearity. The recovery study was performed in triplicate.

Limit of detection and limit of quantitation (LoD & LoQ)

The Y-intercept (standard deviation) and mean slope were replaced in the equation to compute them in compliance with ICH recommendations for DAPA and METO.

Robustness

The parameter was measured by modifying the flow rate (± 0.1 mL/min), temperature (± 5°C) and organic phase content (± 2%). Three assessments were performed using METO (100 µg/mL) and DAPA (20 µg/mL). The outcomes were computed.

Forced degradation studies

These investigations, which characterize the stability of the pharmaceutical material under various stress situations, are useful in figuring out the best storage settings. Sample stock solution was utilized for the degradation studies. For acid and base degradation, the sample solution was refluxed at 60°C for 2 hours with 2 N HCl and 2N NaOH. For oxidative degradation, a sample was refluxed utilizing 3% hydrogen peroxide at 60°C for 2 hours. The samples underwent thermal degradation for 6 hours in a hot air oven (60°C) and photolytic degradation for 24 hours at 340 nm in a UV chamber. Dilution was done for all the degraded samples utilizing diluent to achieve 20 µg/mL DAPA and 100 µg/mL METO. After injecting 30 µL of solution, the chromatogram was recorded to ascertain the stability of the samples.

Greenness assessment

Utilizing the GAPI tool and AGREE calculator, the proposed and reported method's greenness was evaluated. By taking into account the 15 characteristics, such as sample preparation, volume and health risks associated with chemicals and solvents, instruments, waste quantity, and processing, GAPI might be used for a variety of analytical techniques. The three colors (yellow, green and red) are representations of the levels of environmental degradation. The AGREE metric system applies significance-based metrics to assess how environmentally conscious analytical processes are. AGREE is a simple-to-use application with user-friendly software that has been enhanced with the addition of 12 fundamental concepts for assessing greens, weight assignment for flexible working, and a color pictogram output that is easy to understand and highlights strong and weak elements. The value of the Greens Analytical score, which is rounded to two decimals and displayed in the center of the graph, varies from 0.0 to 1.0.

Results

Method Development

Chromatographic parameters: preparation of eluent, column, wavelength of estimation, flow rate and temperature of the column were adjusted during the

technique development process to increase the efficiency of the chromatographic system. The developmental trials were carried out in accordance with literature review and physicochemical properties of both drugs were also taken into consideration. Different columns like Nucleosil C18 (150 mm × 4.6 mm, 5 µm), Cosmosil-MS-5 C18 (250 × 4.6 mm, 5 µm) were tried. The column (Cosmosil-MS-5 C18 (250 × 4.6 mm, 5 µm) was chosen during technique optimization based on the retention period, tailing factor, theoretical plates, and peak shape. Different solvents like acetonitrile, water, methanol, and KH_2PO_4 (20 mM) in varying ratios were investigated in order to obtain sufficient retention and resolution of DAPA, METO and its generated degradation products under a variety of forced degradation circumstances. Eluent containing Methanol: 20 mM potassium dihydrogen phosphate (70:30% v/v) pumped at 25°C at 1.5 mL/min in isocratic mode was finalized as it provided the ideal polarity for DAPA and METO peak migration, segregation, and resolution. The overlapped spectra revealed an iso-absorptive point at 223 nm, which was finalized as a wavelength of detection (Fig. 2). The R_t of METO and DAPA were 2.646 and 11.611 minutes, correspondingly, confirming that METO (log P: 1.76) was more polar over DAPA (log P: 2.7).

Method Validation

System suitability

Since the chromatographic system is a crucial part of the analytical process, its reproducibility was examined using the characteristics of system appropriateness and system repeatability (Table 1). Every prerequisite (resolution > 2, theoretical plates > 2000, tailing factor < 2) for system appropriateness was satisfied and all values are within acceptable limits.

Specificity

It was confirmed from Fig. 3, that no more peaks were seen at either drug's retention time and the current approach was specific for the concurrent quantitation of both drugs in laboratory prepared mixture.

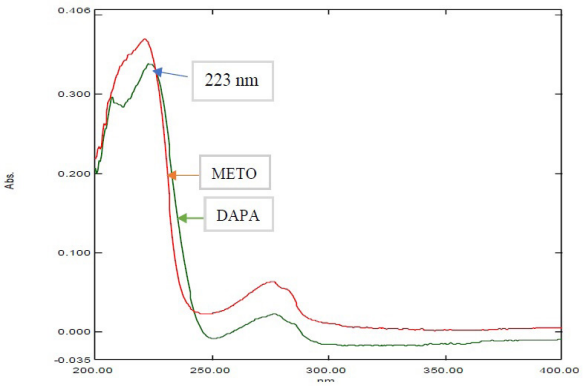

Fig. 2: Overlay UV spectra of DAPA and METO

Table 1: System suitability data

Parameters	*DAPA (Mean ± SD)*	*METO (Mean ± SD)*
Retention time (min)	11.621 ± 0.12	2.72 ± 0.04
Theoretical plates	8712.16 ± 115.7	3481.83 ± 36.43
Tailing factor	1.05 ± 0.004	1.41 ± 0.005
Resolution	26.1 ± 0.09	-

*(n = 6)

Linearity

The method was linear over the concentration range 10 to 30 µg/mL with R^2 of 0.9993 for DAPA and 50 to 150 µg/mL with R^2 of 0.9989 for METO. The outcomes are tabulated in Table 2.

Precision

Intermediate precision (intra-day and inter-day) and repeatability were employed to evaluate the suggested method's precision. The precision studies' percent RSD was determined to be less than 2%. For repeatability, the %RSD of DAPA and METO was determined to be 0.60 and 0.71, correspondingly. The outcomes for intraday and interday precision are represented in Table 3.

Accuracy

Evaluating the recovery performance of the method is equivalent to demonstrating its correctness. Three levels

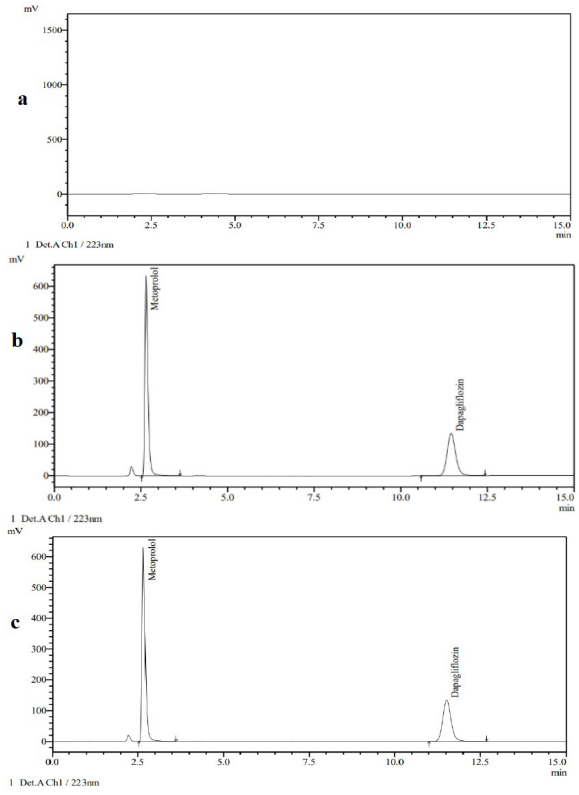

Fig. 3: Chromatogram of (a) Placebo, (b) Standard solution of METO and DAPA and (c) Sample solution of METO and DAPA

Table 2: Linear regression data of DAPA and METO

Parameters	*DAPA*	*METO*
Linearity range (µg/mL)	10–30	50–150
R^2	0.9993	0.9989
Slope	134392	4725
Intercept	113505	123017

of known drug concentrations (80, 100, and 120%) were spiking the placebo to show the accuracy of the existing methodology. The mean %recovery was 99.92 and 100.24 for DAPA and METO, correspondingly. The outcomes are tabulated in Table 4.

LoD and LoQ

DAPA was found to have LoD and LoQ of 0.49 and 1.48 correspondingly and METO was found to have LoD and LoQ of 0.59 and 1.79 correspondingly.

Assay

Using the average of six determinations, the mean %assay for DAPA and METO was found to be 100.5 and 99.8%, respectively. The outcomes are presented in Table 5.

Robustness

To assess the robustness, a few chromatographic conditions, such as temperature (± 5°C), organic phase (± 2%) and flow rate (± 0.1 mL/min) were varied, and the percent RSD values were computed. %RSD for peak area was less than 2%, demonstrating the method's robustness and shown in Table 6.

Forced degradation studies

This study aims to assess the stability of the drugs under particular stress scenarios. DAPA and METO did not interfere with the degradation products when different stimuli for stress studies were applied, according to the HPLC data (Figs 4 and 5). Evaluating the existence of impurities with the primary analyte peak is a crucial step in the SIM validation. Peak purity results for greater than 0.990 show that the DAPA and METO peaks are homogenous under all evaluated stress environments, proving the specificity. The %degradation and peak purity when drugs are exposed to different stress stimuli is depicted in Table 7. The outcomes of the stress study indicate that the drugs were stable under conditions of thermal and photolytic; nevertheless, they become unstable under conditions of acid, alkali and oxidative stress. The maximum allowable forced degradation, according to ICH rules, is less than 20%. Less than 20% degradation of DAPA and METO in the current approach indicates the stability-indicating method.

Greenness assessment

The result of the GAPI diagram is shown in the Fig. 6 (A). It is composed of five pentagrams, each representing for a distinct stage of the analytical method, such as reagent and solvent usage, instrumentation, sample collection, sample preparation, and analytical method goal. No red fields, four yellow fields, and greater numbers of green fields (i.e., 11) were obtained using the GAPI tools for the current method. Yellow pentagrams correspond to the

Table 3: Intermediate precision study of DAPA and METO

DRUG	*Conc. (µg/mL)*	*Intra-day precision*		*Inter-day precision*	
		Mean peak area ± SD	*%RSD*	*Mean peak area ± SD*	*%RSD*
DAPA	10	1178281 ± 10910.03	0.93	1175080 ± 2613.09	0.22
	20	2140366 ± 27082.87	1.27	2143928 ± 23813.58	1.11
	30	3382216 ± 12070.97	0.36	3390825 ± 10377.47	0.31
METO	50	1932053 ± 3762.74	0.19	1931707 ± 2218.12	0.11
	100	3786580 ± 13757.33	0.36	3779863 ± 11350.30	0.30
	150	5707679 ± 12939.31	0.23	5707983 ± 25363.1	0.44

*(n = 3)

Table 4: Accuracy data for DAPA and METO

Drug	*Level (%)*	*Conc. added (µg/mL)*	*Conc. pecovered (µg/mL)*	*Mean %Recovery ± SD**	*%RSD*
DAPA	80	16	16.1	100.19 ± 0.655	0.66
	100	20	20.2	100.56 ± 0.289	0.29
	120	24	23.8	99.01 ± 0.265	0.27
METO	80	80	80.9	101.41 ± 0.625	0.62
	100	100	99.8	99.32 ± 0.289	0.30
	120	120	119.9	99.97 ± 0.231	0.23

*(n = 3)

Table 5: Assay data of DAPA and METO

Parameters	*DAPA*	*METO*
Label claim (mg)	10	50
%Label claim (Mean ± SD)*	100.5 ± 0.81	99.8 ± 0.42
%RSD	0.78	0.44

*(n = 3)

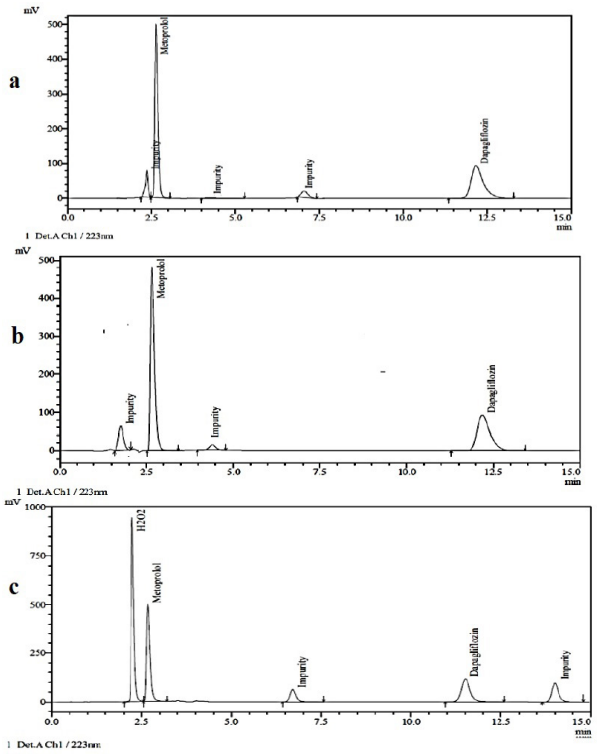

Fig. 4: Chromatogram of degradation (a) Acid, (b) Alkaline and (c) Oxidative

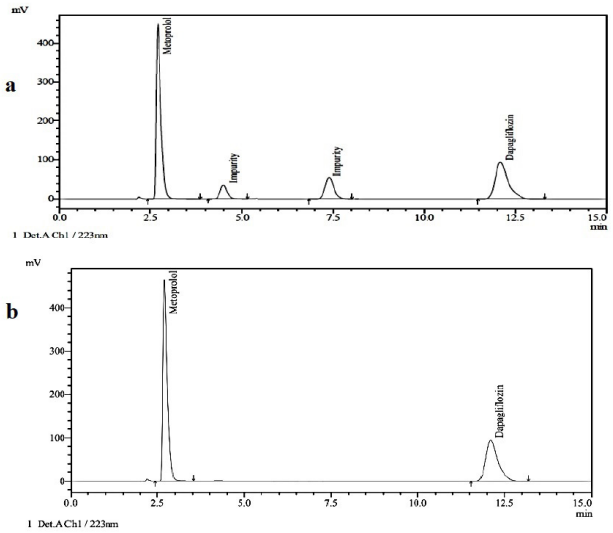

Fig. 5: Chromatogram of degradation (a) Thermal and (b) Photolytic

type of process, which involves waste generation and filtering steps. The numerical value (0.81) produced from AGREE was used to reflect the environmental friendliness of the developed approach (Fig. 6 (B)). The outcomes of GAPI and AGREE pictogram are represented in Fig. 7(A) and 7(B). Greater numbers of yellow fields (i.e., 6), nine green fields, and no red fields were obtained using the GAPI tools for the reported method. More yellow fields in a reported method is an outcome of use of a toxic reagent (acetonitrile having a high NFPA health hazard score). The AGREE score of the reported method was found to be 0.73. As the waste generation in a reported method is less, so section 7 of the AGREE score was yellow as compared to the developed method. Section 11 of the AGREE score for reported shows a red color due to the use of acetonitrile. When it comes to the environment, methanol is thought to be preferable to acetonitrile. Because of the severe toxicity and explosive nature of acetonitrile, Section 12 emphasizes the significance of operator safety.

A score of 0.81 indicates a high level of compliance with the GAC requirements, which include minimizing or eliminating hazardous compounds, decreasing waste creation, increasing energy efficiency, and enhancing elements related to health and safety. It exhibits resolute dedication to ecologically responsible behavior and sustainability. The current approach was found to be environmentally friendly, utilizing green assessment tools to gauge how environmentally friendly it is.

Table 6: Robustness study for DAPA and METO

Parameter	Variation	Retention time* (min)		Mean peak area* ± SD*		%RSD	
		DAPA	METO	DAPA	METO	DAPA	METO
Temperature (± 5°C)	20	11.82	2.69	2207297 ± 1463.78	3881654 ± 1417.56	0.1	0.04
	30	10.31	2.67	2209467 ± 764.35	3889524 ± 1286.81	0.03	0.03
Flow rate (± 0.1 mL/min)	1.4	12.26	2.96	2448171 ± 607.001	4303250 ± 1903.87	0.02	0.04
	1.6	10.10	2.43	2005689 ± 3099.10	3526104 ± 5208.64	0.15	0.15
Organic phase (± 2 %v/v)	32:68	13.05	2.75	2218274 ± 2471.21	3896514 ± 2665.70	0.1	0.07
	28:72	9.43	2.64	2225924 ± 1813.64	3921186 ± 4862.46	0.08	0.12

*(n = 3)

Table 7: Degradation (Forced) data

Degradation stimuli	*%Degradation*		*%Peak purity*	
	DAPA	*METO*	*DAPA*	*METO*
Acid	18.8	8.6	0.9994	0.9996
Base	8.5	13.7	0.9996	0.9995
Oxidative	11.8	10.5	0.9995	0.9996
Thermal	10.2	6.8	0.9998	0.9998

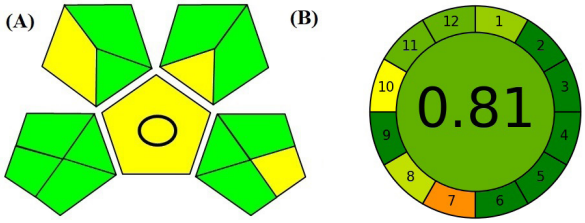

Fig. 6: GAPI (A) and AGREE (B) pictogram for current approach

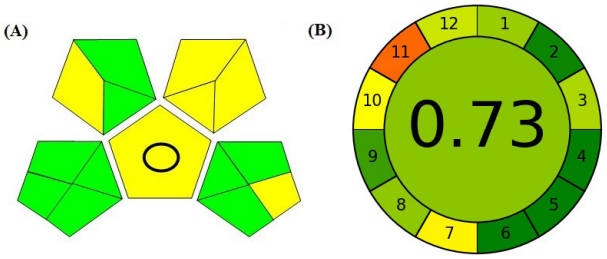

Fig. 7: GAPI (A) and AGREE (B) pictogram for reported approach

Additionally, the current approach outperforms the reported method in terms of specificity because it is stable and provides an effective separation of the stated analytes from their degradation products. Moreover, the current approach was assessed for its eco-friendliness by utilizing AGREE and GAPI tools. Moreover, the current approach and reported method were assessed for their eco-friendliness with the application of AGREE and GAPI tools. Using the GAPI tools for the reported method, more green fields (i.e., 11), four yellow fields, and no red fields were obtained. In comparison to the reported method (32), the new strategy proved to be more environmentally friendly based on the results for both tools.

Conclusion

In this study, a robust, linear, precise and accurate stability indicating RP-HPLC approach for the concurrent quantitation of DAPA and METO in a laboratory-prepared mixture is provided. A database search indicated that no efforts have been undertaken to far to create a thorough stability indication assay protocol using any analytical technique for this binary mixture. Good resolution and separation of drugs and degradants is a major benefit of this study. When this approach is used to analyze the blend, it is demonstrated that, nonetheless, the excipients or the degradation products affect the analytical result. According to the results obtained in different stress studies, both drugs were vulnerable to oxidative, acid and alkaline stimuli. While, they are stable in photolytic as well as thermal stimuli. Regression values, percent RSD, and standard deviations that are appropriate for simultaneous estimation of both pharmaceuticals in a synthetic blend give it versatility and usefulness. Thus, DAPA and METO can be routinely estimated in quality control laboratories using the proposed and proven stability-indicating approach. Using the AGREE and GAPI tools, the suggested approach was environmentally friendly. This field of investigation implies that the approach that has been developed is adhering to environmentally sustainable criteria.

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